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<p>(21) International Application Number: PCT/US97/14201</p> <p>(22) International Filing Date: 15 August 1997 (15.08.97)</p> <p>(30) Priority Data:</p> <table border="0"> <tr> <td>60/024,038</td> <td>16 August 1996 (16.08.96)</td> <td>US</td> </tr> <tr> <td>60/031,110</td> <td>14 November 1996 (14.11.96)</td> <td>US</td> </tr> <tr> <td>60/037,981</td> <td>13 February 1997 (13.02.97)</td> <td>US</td> </tr> <tr> <td>Not furnished</td> <td>5 August 1997 (05.08.97)</td> <td>US</td> </tr> </table> <p>(71) Applicant: CORTECH, INC. [US/US]; 6850 North Broadway, Denver, CO 80221 (US).</p> <p>(72) Inventors: WHALLEY, Eric, T.; 15955 West 77th Place, Arvada, CO 80007 (US). RELTON, Jane, K.; 11B Baalbec Road, Islington, London N5 (GB).</p> <p>(74) Agents: BURKE, John, E.; Cushman Darby &amp; Cushman Intellectual Property Group of Pillsbury Madison &amp; Sutro, 1100 New York Avenue, N.W., Washington, DC 20005 (US) et al.</p>	60/024,038	16 August 1996 (16.08.96)	US	60/031,110	14 November 1996 (14.11.96)	US	60/037,981	13 February 1997 (13.02.97)	US	Not furnished	5 August 1997 (05.08.97)	US	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report.</i></p>
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<p>(54) Title: METHODS TO TREAT CEREBRAL ISCHEMIC INJURY AND OTHER NEURONAL DISEASES</p> <p>(57) Abstract</p> <p>The present invention relates generally to the use of antagonists which selectively affect the bradykinin 2 receptor (B<sub>2</sub>) and/or agonists which selectively affect the B<sub>1</sub> receptor, to treat cerebral ischemic injury, whether due to stroke or other causes, and in particular to striatal ischemic injury and cortical ischemic injury. The present invention also relates to using B<sub>2</sub> antagonists and/or B<sub>1</sub> agonists to treat Parkinson's disease and Huntington's disease. The B<sub>2</sub> antagonist and/or B<sub>1</sub> agonist chosen can be used alone or together as the therapeutic agent or in combination with other therapeutic agents.</p>													

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## Methods to Treat Cerebral Ischemic Injury and other Neuronal Diseases

5 The following patents or patent application publications pertaining to B<sub>2</sub> antagonists and their synthesis are relevant:

US 4,693,993; WO 94/06453; WO 91/09055; EP 552,106; EP 370,453-A2; EP 413,277-B1; WO 93/11789; EP 564,972; WO 94/08607; EP 578,521; WO 94/19372; WO 94/11021; US 5,228,725; EP 596,406-A1; WO 95/07294 and EP 622,361-A1.

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### BACKGROUND OF THE INVENTION

#### Field of the Invention

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The present invention relates generally to the use of antagonists which selectively affect the bradykinin 2 receptor (B<sub>2</sub>) or agonists which selectively affect the bradykinin 1 receptor (B<sub>1</sub>) to treat cerebral ischemic injury, whether due to stroke or other causes, and in particular to striatal ischemic injury and cortical ischemic injury. The present invention also relates to using B<sub>2</sub> antagonists and/or B<sub>1</sub> agonists to treat Parkinson's disease and Huntington's disease or other neuronal diseases in which the striatum or fine motor control is affected. The B<sub>2</sub> antagonist and/or B<sub>1</sub> agonists chosen can be used alone or together as the therapeutic agent or alone and together in combination with other therapeutic agents.

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### Description of the Background

Stroke affects 1.5 million people per year worldwide. Disabilities associated with stroke, in the event that the patient survives, include the loss of the ability to communicate, ambulate, coordinate or reason. Damage to the cortical region of the brain usually results in impaired speech and the loss of limb control, among other problems. Previous treatments for stroke, or compounds in pre-clinical or clinical development for stroke, have provided mainly cortical protection or are aimed at reducing edema (abnormal accumulation of serous fluid in the brain cavity). Damage to the striatum results in impairment or loss of fine motor skills and has proven difficult to treat. No treatments to date are effective at both the striatum and the cortex. The present methods provide such treatments.

Cerebral ischemic injury due to stroke has been attributed to the depolarization of neurons upon energy deprivation which causes impaired glutamate receptor function and can result in mitochondrial injury, enzyme activation/inactivation, cellular swelling, and excitotoxicity. Therefore, pharmacological strategies for stroke include: blockade of glutamate receptor function using channel blockers, NMDA or AMPA receptor antagonists; free radical scavengers to ameliorate reperfusion injury; voltage-gated channel blockers using sodium and calcium channel blockers or calcium channel modulators; anti-inflammatory agents such as white blood cell adhesion blockers; anticoagulation and thrombolysis; neurotrophins; potentiation of inhibitory neurotransmitters and various other strategies. All of these studies are cited in a review article by Koroshetz and Moskowitz, 17 *TIPS* 227 (1996).

In order to fully understand the present invention, a brief discussion of the role of bradykinin in the etiology and pathophysiology of neuronal damage and brain edema is necessary. Bradykinin causes neuronal damage and injury (Francel, 9 *J. Neurotrauma* S27 (1992), Ellis, 4 *New Trends Lipid Mediators Res.* 129 (1990), is a mediator in vasogenic brain edema (Unterberg *et al.*, *Recent Progress in the Study and Therapy of Brain Edema* 175 (1984), Unterberg and Baethmann, 61 *J. Neurosurg.* 87 (1984), and augments the progression of ischemic brain edema (Kamiya *et al.*, 24 *Stroke* 571 (1993). Kininogen, which is known to be the precursor for bradykinin, is consumed during ischemia and reperfusion of the brain in rats and in humans undergoing neurosurgical treatment for stenotic and occlusive carotid damage. (Makevnina *et al.*, 27(8) *Brazilian J. Med. & Bio. Res.* 1955 (1994).

However, the role of bradykinin antagonists in ischemia is not predictable from organ to organ. Bradykinin antagonists have been shown to attenuate the vascular response to hyperventilation following brain injury in rabbits (Ellis, 4 *New Trends Lipid Mediators Res.* 129 (1990)), reduce brain edema in rats following cold lesion trauma and reduce brain edema in man following traumatic brain injury (Rodell, 30 *Immunopharmacology* 279 (1996)). In contrast, in models of myocardial infarction and cardiac ischemia-reperfusion injury both bradykinin B<sub>2</sub> and B<sub>1</sub> receptor antagonists worsen the condition whereas the B<sub>2</sub> agonist bradykinin and the B<sub>1</sub> agonist, des-Arg<sup>9</sup>-BK, are protective. Chahine *et al.*, 108 *Br. J. Pharmacol.* 318 (1993); Linz *et al.*, 47 *Pharm. Rev.* 25 (1995); Parrart *et al.*, 73 *Can. J. Physiol. Pharmacol.* 837 (1995).

An EPO patent application publication, WO 95/07294, discloses bradykinin B<sub>1</sub> and B<sub>2</sub> receptor antagonists. In that application, the use of the compounds for stroke is mentioned, but no data are shown. Likewise, in U.S. Application bradykinin antagonists are mentioned in conjunction with stroke. Both disclosures are speculative in nature and do not provide data.

A B<sub>1</sub> antagonist has also been shown to reverse the protective effect of a B<sub>2</sub> antagonist on survival in porcine endotoxic shock. Siebeck et al, Immunopharmacology. However, no clear understanding of B<sub>1</sub> role in normal physiology has been reached.

### SUMMARY OF THE INVENTION

It is an objective of the present invention to provide a method to treat cerebral ischemic injury using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist, alone or together as the therapeutic agent or alone or together in combination with other therapeutic agents.

It is a further object to provide a method to treat stroke using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist, alone or in combination with another treatment.

It is therefore an object to provide a method to treat striatal ischemic injury using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist, alone or in combination with another treatment.

It is also therefore an object to provide a method to treat cortical ischemic injury using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist, alone or in combination with another treatment.

5 It is yet another object to provide a method to treat neuronal diseases using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist.

It is therefore an object to provide a method to treat Huntington's or Parkinson's disease, using a B<sub>2</sub> antagonist and/or a B<sub>1</sub> agonist, alone or in combination with another treatment.

10 It is also an object to provide a method to treat reperfusion injury using a B<sub>2</sub> antagonist alone or in combination with another treatment.

Other objects and features of the present invention will be apparent from the following detailed description.

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### **BRIEF DESCRIPTION OF THE DRAWINGS**

In Figures 1-8, all compounds were given by mini osmotic pump subcutaneously 30-45 minutes before permanent occlusion of the middle cerebral artery, and for the duration of the  
20 experiment (24 hours).

Figure 1: The effect of the bradykinin B<sub>2</sub> receptor antagonist CP-0597 on total infarct volume in

the rat 24 hours following permanent occlusion of the middle cerebral artery. This figure shows a dose-dependent, protective effect of CP-0597 compared to control untreated rats. Note that the 30 ng/kg/min dose of CP-0597 used a separate group of control rats (b).

5 Figure 2: The effect of the bradykinin B<sub>2</sub> receptor antagonist, CP-0597 on striatal infarct volume in the rat 24 hours following permanent occlusion of the middle cerebral artery. This figure shows a dose-dependent, protective effect of CP-0597 compared to saline treated control rats.

10 Figure 3: The effect of the combined B<sub>2</sub>/B<sub>1</sub> receptor antagonist B9430 on total infarct volume in the rat 24 hours following permanent occlusion of the middle cerebral artery. This shows that blocking both B<sub>2</sub> and B<sub>1</sub> receptors provided no protection and may have worsened the damage compared to saline-treated control rats.

15 Figure 4: The effect of a B<sub>1</sub> antagonist (B-9858) alone and in combination with the B<sub>2</sub> antagonist, CP-0597, on total infarct volume 24 hours following permanent occlusion of the middle cerebral artery in the rat. This shows that a B<sub>1</sub> antagonist alone had no effect on infarct volume and when given in combination with CP-0597, reversed the protective effect of CP-0597 alone (see Figure 1) and may have worsened the damage compared to saline-treated controls.

20 Figure 5: The effect of the B<sub>2</sub> antagonists HOE140 and NPC17731 on total infarct volume in the rat 24 hours following permanent occlusion in the rat. Both these B<sub>2</sub> receptor antagonists provided protection against damage compared to controls.



Figure 6: The effect of the bradykinin B<sub>2</sub> receptor antagonists HOE140 and NPC17731 on striatal infarct volume 24 hours following permanent occlusion of the middle cerebral artery in the rat. Both these B<sub>2</sub> receptor antagonists provided significant protection against striatal damage compared to controls.

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Figure 7: The effect of the NMDA antagonist MK801 on total infarct volume in the rat 24 hours following permanent occlusion of the middle cerebral artery in the rat. MK801 provided significant protection from damage compared to control rats.

10 Figure 8: The effect of the NMDA antagonist MK801 on striatal infarct volume 24 hours following permanent occlusion in the rat. MK801 provided no protection against striatal damage compared to saline-treated controls.

Figure 9: The effect of the bradykinin B<sub>2</sub> receptor antagonist CP-0597 on a) total, b) cortical and  
15 c) striatal infarct volume 24 hours following permanent occlusion of the middle cerebral artery. In this experiment the compound was given 30 to 40 minutes after occlusion of the artery and represents post-treatment. CP-0597 provided significant protection of striatal damage but not cortical damage.

20 Figure 10: Structures of CP-0597, HOE140 and NPC17731.

Figure 11: Effect of the B<sub>2</sub> antagonist CP-0597 on behavioral score before and after reversible

MCAO in the rat. Treatment with CP-0597 significantly improved mean behavioral score compared to vehicle treated controls measured 24 hours after reversible MCAO. ( $P < 0.01$ ).

Figure 12: Effect of CP-0597 on change in body weight 24 hours after reversible MCAO in the rat. Mean drop in body weight over the experimental period was significantly less ( $p < 0.01$ ) in CP-0597 treated rats compared to vehicle controls.

Figure 13: Dorsal view of rat brain indicating sections used in the rMCAO model of stroke. Illustrates a dorsal view of the rat brain indicating the points at which sections were taken for histological analysis of brain damage.

Figure 14: Coronal sections of rat brain 24 hours post rMCAO. Distribution of brain damage on H&E stained brain sections (A-E) from rats treated with either vehicle or CP-0597.

Figure 15: Effect of CP-0597 on edema: percent increase in hemisphere size. The percentage increase in hemisphere size observed after reversible MCAO was significantly reduced in all brain sections (A-E) from rats treated with CP-0597 compared to vehicle treated controls ( $p < 0.05$ ).

Figure 16: Effect of CP-0597 on absolute infarct area 24 hours after rMCAO. The area of absolute brain infarction was reduced in all brain sections (A-E) in rats treated with CP-0597 compared to vehicle treated controls.

Figure 17: Effect of CP-0597 on absolute infarct volume 24 hours after rMCAO. Total absolute infarct volume was calculated as the area under the curve of infarct areas on sequential brain sections plotted against their respective stereotaxic co-ordinates. Mean absolute infarct volume was significantly reduced by 60% ( $p < 0.001$ ) in rats in CP-0597 treated rats compared to vehicle treated controls. Cortical and subcortical regions were protected by 63% and 43% respectively.

Figure 18: Effect of CP-0597 on infarct area 24 hours after rMCAO. The area of pallor on each brain section was sketched onto stereotaxic maps and extent of infarction quantified from the maps to adjust for hemispheric swelling. The area of infarction calculated in this manner was reduced in all brain sections (A-E) in rats treated with CP-0597 compared to vehicle treated controls.

Figure 19: Effect of CP-0597 on infarct volume 24 hours after rMCAO. Total adjusted infarct volume was calculated as above. Infarct volume was significantly reduced by 57% ( $p < 0.001$ ) in CP-0597 treated rats compared to vehicle treated controls. Cortical and subcortical regions were protected by 60% and 49% respectively.

Figure 20: Areas of rat brain sampled for neuronal damage. Illustrates the specific regions of the brain sample for neuronal counts. The number of necrotic neurons were counted at identical sites on section C of H&E stained brain sections from vehicle and CP-0597 treated animals.

Figure 21: Neuronal counts, Section C, Area II. Shows photomicrographs (20X magnification)

of H&E stained C sections (area II, parietal cortex) from a) a sham operated control, b) a vehicle treated rat 24 hours after rMCAO and c) a CP-0597 treated rat 24 hours after rMCAO.

Figure 22: Effect of CP-0597 on number of dead neurons per 80X field. The number of necrotic neurons in areas I-IV was significantly reduced in CP-0597 treated rats compared to vehicle treated controls.

Figure 23: Identification of bradykinin B<sub>1</sub> receptors in rat brain following a) one hour of ischemia and b) 24 hours of reperfusion. Note B<sub>1</sub> receptors (black stain) in b) associated with specific neurons.

### **Detailed Description Of The Invention**

It has been newly discovered by the present inventors that antagonism of the B<sub>2</sub> receptor and/or agonism of the B<sub>1</sub> receptor in the brain results in protection from damage due to overproduction of the naturally-occurring peptide bradykinin. Until the present disclosure, it was unclear what the B<sub>2</sub> or B<sub>1</sub> receptors' role in pathological states associated with bradykinin was. The confusion was primarily because no experimentation had been done, and no conclusions had been made, but also because conflicting evidence of bradykinin's role in ischemia was present. Since the inventors have clarified the role of B<sub>2</sub> antagonists and B<sub>1</sub> agonists in neuronal pathologies, the present invention is drawn to methods to treat neuronal pathologies using B<sub>2</sub> antagonists and/or B<sub>1</sub> agonists as therapeutic agents.

Newly discovered and clinically significant is the ability of B<sub>2</sub> antagonists and/or B<sub>1</sub> agonists to treat ischemic injury in both the striatum and the cortex. Previously described treatments have resulted in predominately cortical protection when, in the pathology of stroke, both the cortex and striatum are affected. Thus, previous treatments have inadequately addressed the complete extent of damage because they have neglected treatment of the striatum. It is therefore surprising and unexpected to find a class of compounds which will treat both cortical damage as well as striatal damage. The present invention provides a treatment for stroke which improves dramatically over those previously available.

#### B<sub>2</sub> Antagonists

B<sub>2</sub> antagonists are generally separated into two categories: peptide-based and non-peptide-based. Those in the art are aware of the available B<sub>2</sub> antagonists, and are also aware of minor modifications of the known B<sub>2</sub> antagonists which would result in active compounds. Moreover, it is assumed that many additional peptide and non-peptide B<sub>2</sub> antagonists will be invented in the future. For the purposes of the present disclosure, any compound which is shown to have selective activity at the B<sub>2</sub> receptor would be useful in the invented methods. "Selective for the B<sub>2</sub> receptor," as used herein shall mean any compound in which the B<sub>2</sub>/B<sub>1</sub> K<sub>i</sub> ratio is greater than 100. However, ratios in the range of 500 to 1000 are preferred. Most preferred is a compound with a B<sub>2</sub>/B<sub>1</sub> ratio greater than 1000.

Peptide-based B<sub>2</sub> antagonist compounds are listed, for example, in the following

publications: Cheronis *et. al.*, 35 *J. Med. Chem.* 1563 (1992); Kyle & Burch, 2 *Curr. Opin. Invest. Drugs* 5 (1993); Hock *et al.*, 102 *Br. J. Pharmacol.* 769 (1991); Hock *et al.*, 102 *Br. J. Pharmacol.* 774 (1991); Burke *et. al.*, 5(4) *Exp. Opin. Ther. Patents* 331 (1995), and others. All of these compounds would be useful in the presently disclosed methods.

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Non-peptide and peptoid B<sub>2</sub> antagonist compounds include those mentioned by: Sawutz *et. al.*, 91 *Proc. Nat'l Acad. Sci.* 4693 (1994); Lam *et. al.*, 52 *Tetrahedron* 481 (1996); Inamura *et al.*, Characterization of FR 173657, a novel non-peptide BK<sub>2</sub> antagonist, *in vitro* and *in vivo* studies, *Proceedings-Peptide Receptors Symposium, Montreal* (1996); Kyle *et al.* WO 95/07294 (1995) and others. All of these compounds would be useful in the presently-disclosed methods.

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Moreover, many treatments for neural pathologies are already known. Combinations of B<sub>2</sub> antagonists with these treatments are considered within the scope of the present invention. Two recent reviews provide examples of the types of treatments which could be combined with a B<sub>2</sub> antagonist pursuant to the present invention. Koroshetz & Moskowitz, 17 *TIPS* 227 (1996) Barinaga, 272 *Science* 664 (1996). For the purposes of this disclosure, such treatments are defined as "adjuvant(s)".

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Most of the above agents are effective in animal models of stroke, their mechanism of action being predominantly via protection of cortical damage or reducing inflammation/edema. None of the above agents have significant effects against striatal damage. As disclosed in the present invention, bradykinin B<sub>2</sub> antagonists reduce total infarct volume with the major effect

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being protection of striatal rather than cortical damage. Bradykinin antagonists also reduce brain edema in man. Thus, the combination of a bradykinin B<sub>2</sub> antagonist with an adjuvant is synergistic and provides protection against both cortical and striatal damage.

5           Lastly, assays for determining if a compound is a B<sub>2</sub> receptor antagonist are known. The rat and human B<sub>2</sub> receptors have been cloned and an assay based on the injected RNA in frog oocytes has been characterized. See, Burke *et al.*, *Molecular Biology and Pharmacology of Bradykinin Receptors* 19 (1993). Moreover, the classical assay for determining B<sub>2</sub> activity, the guinea pig ileum assay, is still applicable. The classical assay for B<sub>1</sub> activity, the rabbit aorta  
10 smooth muscle assay, is reviewed and compared to other assays in Burke *et al.*, *Molecular Biology and Pharmacology of Bradykinin Receptors* 6 (1993).

#### B<sub>1</sub> Agonists

Examples of the peptide-based B<sub>1</sub> agonists can be found in the following citations:

15 Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-  
20 272.

Peptide and peptide-based Bradykinin B<sub>1</sub> agonists can be made, identified and assayed as described in the references. Bradykinin B<sub>1</sub> agonists can also be purchased from a variety of commercial sources including Phoenix Pharmaceuticals, Inc. (Mountain View, California), Sigma Chem. Co. (St. Louis, Missouri), Bachem (Torrence, California) and Peninsula Labs (Belmont, California).

The relevance of animal models in stroke have been reveiwd by Hunter *et al.*, 6 *TIPS* 123 (1996). The rat model of permanent middle cerebral artery occlusion mimics the human condition of stroke and is a standard model used for the evaluation of compounds believed to be of potential therapeutic benefit for the treatment of this disorder. Tamura *et. al.*, 1(1) *J. Cereb. Blood Flow Metabol.* 53 (1981); Garcia *et. al.*, 26(4) *Stroke* 627 (1995).

The synthesis of the peptides useful in this invention including derivation, activation, and coupling of protected amino acid residues, and their purification, and the analytical methods for determining identity and purity are included in the general body of knowledge of peptide chemistry, as described in Houben Weyl Methoden der Organischen Chemie (1974) Vol. 16, parts I & II for solution-phase synthesis, and in Solid Phase Peptide Synthesis, (1984), by Stewart and Young for synthesis by the solid-phase method of Merrifield.

Any chemist skilled in the art of peptide synthesis can synthesize the peptides useful in this invention by standard solution methods or by manual or automated solid phase methods.



The method of treatment according to this invention involves administering internally or topically to subject an effective amount of active compound. Doses of active compounds in the inventive method are an efficacious, nontoxic quantity selected from the range of 0.01 to 500 mg/kg of active compound, preferably 0.1 to 50 mg/kg. Persons skilled in the art using routine clinical testing are able to determine optimum doses for the particular ailment being treated. The desired dose is administered to a subject from 1 to 6 or more times daily, intravenously, orally, rectally, parenterally, topically, or by inhalation. The desired dose may also be given by continuous, intravenous infusion; this is the preferred mode of administration.

In parenteral administration of the B<sub>2</sub> antagonists and/or B<sub>1</sub> agonists pursuant to this invention, the compounds may be formulated in aqueous injection solutions which may contain antioxidants, buffers, bacteriostats, etc. Extemporaneous injection solutions may be prepared from sterile pills, granules, or tablets which may contain diluents, dispersing and surface active agents, binders and lubricants which materials are all well known to the ordinary skilled artisan.

In the case of oral administration, fine powders or granules of the compound may be formulated with diluents and dispersing and surface active agents, and may be prepared in water or in a syrup, in capsules or cachets in the dry state or in a non-aqueous suspension, where a suspending agent may be included. The compounds may also be administered in tablet form along with optional binders and lubricants, or in a suspension in water or syrup or an oil or in a water/oil emulsion and may include flavoring, preserving, suspending, thickening, and emulsifying agents. The granules or tablets for oral administration may be coated or other

pharmaceutically acceptable agents and formulations may be utilized which are all known to those skilled in the pharmaceutical art.

Solid or liquid carriers can also be used. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, and water. Ointments and creams are prepared using various well known hydrophilic and hydrophobic bases. Topical reservoirs are suitably prepared using known polymeric materials such as various acrylic-based polymers selected to provide desired release characteristics. Suppositories are prepared from standard bases such as polyethylene glycol and cocoa butter.

## Examples

### Ex.1. Protocol for Permanent Occlusion Model

Groups of rats were anesthetized with halothane and underwent permanent occlusion of the left middle cerebral artery (MCA) or underwent sham surgery. Permanent occlusion was induced by electrocoagulation of the MCA. The rats were allowed to recover and after 24 hours were sacrificed, at which time infarct volume and other parameters were quantified. Infarct size was determined from tetrazolium stained 500 micrometer coronal brain sections.

Groups of animals were pretreated with bradykinin antagonist or vehicle in a blinded fashion. Drug, or saline was delivered subcutaneously via pre-activated mini-osmotic pumps

implanted just before MCA surgery. Doses of 10, 30, 100 and 300 ng/kg/min of CP-0597 were compared with vehicle control given for the 24 hour period.

#### Ex.2 Results for Permanent Occlusion Model

- 5 CP-0597 produced a dose-dependent significant reduction in infarct size in this model of stroke. The NMDA antagonist MK801, given at a dose of 4 mg/kg sec., was also tested in this model. CP-0597 produced an equivalent degree of reduction in infarct size at a total dose of only 0.432 mg/kg over the 24 hour period compared to that seen with 4 mg/kg of MK801.
- 10 CP-0597 produced a dose-dependent reduction in total infarct size in this model of stroke (Figure 1) comparable to that seen with 4mg/kg of MK 801 (figure 7). The effect of CP-0597 was greatest against striatal damage producing a greater than 90% protection compared to controls (Figure 2), with some protection against cortical damage. MK801 produced its protective effect against cortical damage having little to no effect in the striatal region (Figure
- 15 8). This protective effect of CP-0597 against striatal and cortical damage was also seen with two other B<sub>2</sub> antagonists, NPC17731 and HOE140 (Figures 5 & 6). The combined B<sub>2</sub>/B<sub>1</sub> antagonist, B9430 slightly worsened brain damage (Figure 3). A B<sub>1</sub> antagonist alone had no effect and in combination with with the B<sub>2</sub> antagonist CP-0597 slightly worsened damage. This would suggest that B<sub>2</sub> receptors are destructive and that B<sub>1</sub> receptors may be protective. Finally,
- 20 CP-0597 when given 30 to 45 minutes post middle cerebral artery occlusion also provided protection against striatal damage ( Figure 9). These data support the use of a B<sub>2</sub> receptor antagonist and/or a B<sub>1</sub> agonist for stroke/ischemia alone or in combination with an agent that

provides additional protection against cortical damage.

Example 3. Protocol for Reversible Occlusion Model

Reversible MCAO was performed using the rat suture model of Zea Longa, E. *et al*

(1989) *Stroke* 20:84-91 . Briefly, male Wistar rats (270-300g) were anesthetized using halothane

and MCAO produced by the insertion of a 4-0 nylon monofilament into the right external carotid artery, through the internal carotid artery to the origin of the MCA. After 1 hour the filament was retracted to allow reperfusion of the ischemic region. Primed mini-osmotic pumps

containing vehicle or CP-0597 were implanted into the subcutaneous space immediately after MCAO. Pumps released 300ng/kg/min CP-0597 or vehicle at a rate of 8 $\mu$ l/h over the ensuing 24 hour period. Twenty four hours after MCAO animals underwent cardiac perfusion with 0.9%

saline followed by Bouins solution. Fixed brains were sectioned into sequential 1mm blocks (A-E) and 4 $\mu$ m sections and stained with hematoxylin and eosin (H & E). Clinical evaluation of rats

was performed by neurological scoring of forelimb flexion, symmetry of movement and forepaw outstretching (normal=10) and change in body weight over the experimental period. Sequential brain sections (A-E) were assessed histopathologically for percentage change in hemisphere size

of the ischemic hemisphere compared to the contralateral undamaged hemisphere, as a measure of brain edema. Infarct size was measured as the area of pallor on H & E stained brain sections

and quantified as absolute infarct size and infarct size adjusted for edema. Neuronal counts of necrotic neurons were performed in defined cortical and striatal regions of tissue block C

corresponding to the level of the anterior commissure.

#### Example 4. Results of Reversible Occlusion Model

Vehicle treated control rats that underwent MCAO sustained extensive lesions throughout cortical and subcortical regions of the brain, the ischemic hemisphere was markedly swollen and significant behavioral deficits were observed. Treatment with the B<sub>2</sub> receptor antagonist CP-0597 improved behavioral outcome, change in body weight and reduced the extent of infarction and brain edema measured 24 hours after induction of ischemia when compared to vehicle treated control rats. Results are expressed as mean values  $\pm$  sem (n=12 per group). Statistical analysis was performed using Students' t-test (\*\*\*) denotes  $P < 0.001$ , \*\* =  $P < 0.01$ , \* =  $P < 0.05$ .

Neurological score was similar in both treated and untreated animals at the time of reperfusion (1 hour after induction of ischemia), but was significantly improved in CP-0597 treated rats when measured 24 hours after MCAO (vehicle:  $2.58 \pm 0.37$  vs CP-0597:  $6.9 \pm 0.89$ ,  $P < 0.001$ , Figure 1).

The drop in body weight observed over the experimental period was significantly reduced in CP-0597 treated rats ( $-17.7 \pm 3g$ ) compared to vehicle treated controls ( $-28.3 \pm 2.3g$ ,  $P < 0.01$ , Figure 12). Figure 14 illustrates the extensive brain infarction and edema observed in saline treated control brains measured 24 hours after surgery and the dramatic reduction in damage observed after treatment with CP-0597. Quantification of brain damage revealed that vehicle treated rats exhibited profound ipsilateral hemispheric enlargement of between 25 and 40% through brain sections A-E. The percentage increase in hemisphere size was significantly reduced to between 8 and 20% in CP-0597 treated animals (Figure 15). Infarct size was

calculated as both absolute volume as well as volume adjusted for cerebral edema. The area of pallor on each brain section stained with H&E was quantified directly from fixed sections (absolute area) and from sketches made onto stereotaxic maps (adjusted area) for sections A-E. Both absolute and adjusted infarct areas were reduced on all sections analyzed in CP-0597 treated animals compared to vehicle treated controls (Figures 16 and 18). Total infarct volume was calculated as the area under the curve of infarct areas on sequential brain sections plotted against their respective stereotaxic co-ordinates. Mean absolute total infarct volume was significantly reduced by 60% ( $P < 0.001$ ) in CP-0597 treated rats ( $114.1 \pm 30.1 \text{ mm}^3$ ) compared to vehicle treated controls ( $284.9 \pm 17.4 \text{ mm}^3$ ). Subanalysis revealed cortical and subcortical protection of 63% and 43% respectively (Figure 17). Mean adjusted total infarct volume was significantly reduced by 57% ( $P < 0.001$ ) in CP-0597 treated rats ( $88.8 \pm 21.1 \text{ mm}^3$ ) compared to vehicle treated controls ( $204.5 \pm 8.6 \text{ mm}^3$ ) with cortical and subcortical protection of 60% and 49% respectively (Figure 19). Tissue samples taken from brain section C stained with H&E were examined at high magnification (20X) for neuronal counts. The number of necrotic neurons in each of four specifically defined fields (Figure 20) were counted. Treatment with CP-0597 significantly reduced the number of necrotic neurons in the parietal and preoptic cortices and the striatum compared to vehicle treated control animals (Figure 21 and 22).

Treatment with the bradykinin  $B_2$  receptor antagonist CP-0597, after induction of ischemia and before the time of tissue reperfusion significantly protected the brain against ischemic injury in a model of reversible focal cerebral ischemia in the rat. The pathology of this model is clinically representative of the human condition of stroke and the present data suggest that CP-0597 may be of significant benefit in the treatment of this and other ischemia-related

disorders.

Example 5. Evidence for B<sub>1</sub> Agonism

The combined B<sub>1</sub>/B<sub>2</sub> antagonist B9430 at 300 ng/kg/min s.c. had no effect on infarct  
5 volume. The B<sub>1</sub> antagonist B9858 at 300ng/kg/min s.c. alone had no effect on infarct volume  
and when given together with CP-0597 reversed the protective effect of CP-0597.  
Lys0-des-Arg9-Leu8-BK (CP-0298) when given together with CP-0597 also reversed the  
protective effect of CP-0597.

B<sub>1</sub> receptors are not normally present in normal brain. Twenty Four hours after  
10 reperfusion following 1 hour of ischemia (reversible middle cerebral artery occlusion) B<sub>1</sub>  
receptors can be identified in the stroked side of the brain in the cortex and striatum. No B<sub>1</sub>  
receptors can be identified in the non-stroked contralateral hemisphere. This would suggest that  
with time there is an up regulation of the B<sub>1</sub> receptor in the brain following  
ischemia-reperfusion. Hence, this supports the data with the B<sub>1</sub> antagonist reversing the  
15 protective effect of a B<sub>2</sub> antagonist (CP-0597) and that the effect is probably mediated via  
specific B<sub>1</sub> receptors, some of which are located on the neurons in the damaged brain. This  
would suggest that the B<sub>1</sub> receptor is up regulated in response to ischemia and reperfusion in an  
attempt to "protect" the neurons. Hence a bradykinin B<sub>1</sub> agonist would be of benefit following  
ischemia reperfusion of the brain (stroke) and since the receptors are up regulated with time it  
20 would indicate that there is a wide window of opportunity for treatment.

**WHAT IS CLAIMED IS:**

1. A method to treat stroke in a patient in need of such treatment, comprising administering a B<sub>2</sub> antagonist.
2. A method of claim 1, which further comprises administering an adjuvant.
- 5 3. A method of claim 2 wherein the adjuvant is a thrombolytic agent.
4. A method of claim 3 wherein the thrombolytic agent is tissue plasminogen activator.
5. A method of claim 2, wherein the adjuvant is an antagonist of a receptor chosen from the group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.
6. A method of claim 1, wherein the B<sub>2</sub> antagonist is chosen from the group consisting of: CP-  
10 0597, HOE 140 and NPC 17731.
7. A method of claim 6, which further comprises administering an adjuvant.
8. A method to treat cerebral ischemic injury in a patient in need of such treatment, comprising administering a B<sub>2</sub> antagonist.
9. A method of claim 8, which further comprises administering an adjuvant.
- 15 10. A method of claim 9, wherein the adjuvant is a thrombolytic agent.
11. A method of claim 10, wherein the thrombolytic agent is tissue plasminogen activator.
12. A method of claim 9, wherein the adjuvant is an antagonist of a receptor chosen from the group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.
13. A method of claim 8, wherein the B<sub>2</sub> antagonist is chosen from the group consisting of: CP-  
20 0597, HOE 140 and NPC17731.
14. A method of claim 13, which further comprises administering an adjuvant.
15. A method of claim 8, wherein the cerebral ischemic injury is striatal ischemic injury.



16. A method of claim 15, which further comprises administering an adjuvant.
17. A method of claim 16 wherein the adjuvant is a thrombolytic agent.
18. A method of claim 17 wherein the thrombolytic agent is tissue plasminogen activator.
19. A method of claim 16, wherein the adjuvant is an antagonist of a receptor chosen from the  
5 group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.
20. A method of claim 15, wherein the B<sub>2</sub> antagonist is chosen from the group consisting of: CP-0597, HOE 140 and NPC17731.
21. A method of claim 20, which further comprises administering an adjuvant.
22. A method of claim 8, wherein the cerebral ischemic injury is cortical ischemic injury.
- 10 23. A method of claim 22, wherein the B<sub>2</sub> antagonist is chosen from the group consisting of: CP-0597, HOE 140 and NPC17731.
24. A method of claim 23, which further comprises administering an adjuvant.
25. A method to treat the reduction in the ability to perform fine motor skills due to neuronal disease in a patient in need of such treatment, comprising administering a B<sub>2</sub> antagonist.
- 15 26. A method of claim 25, wherein the B<sub>2</sub> antagonist is chosen from the group consisting of: CP-0597, HOE 140 and NPC17731.
27. A method of claim 26, which further comprises administering an adjuvant.
28. A method of claim 25, wherein the neuronal disease is Parkinson's disease.
29. A method of claim 25, wherein the neuronal disease is Huntington's disease.
- 20 30. A method to treat reperfusion injury in a patient in need of such treatment comprising administering a B<sub>2</sub> antagonist.

31. A method to treat stroke in a patient in need of such treatment, comprising administering a B<sub>1</sub> agonist.
32. A method of claim 31, which further comprises administering an adjuvant.
33. A method of claim 32 wherein the adjuvant is a thrombolytic agent.
- 5 34. A method of claim 33 wherein the thrombolytic agent is tissue plasminogen activator.
35. A method of claim 32, wherein the adjuvant is an antagonist of a receptor chosen from the group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.
36. A method of claim 31, wherein the B<sub>1</sub> agonist is chosen from these examples which can be found in the following citations: Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; 10 Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-272.
- 15 37. A method of claim 36, which further comprises administering an adjuvant.
38. A method to treat cerebral ischemic injury in a patient in need of such treatment, comprising administering a B<sub>1</sub> agonist.
39. A method of claim 38, which further comprises administering an adjuvant.
40. A method of claim 39, wherein the adjuvant is a thrombolytic agent.
- 20 41. A method of claim 40, wherein the thrombolytic agent is tissue plasminogen activator.
42. A method of claim 39, wherein the adjuvant is an antagonist of a receptor chosen from the group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.

43. A method of claim 38, wherein the B<sub>1</sub> agonist is chosen from these examples which can be found in the following citations: Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-272.

44. A method of claim 43, which further comprises administering an adjuvant.

45. A method of claim 38, wherein the cerebral ischemic injury is striatal ischemic injury.

46. A method of claim 45, which further comprises administering an adjuvant.

47. A method of claim 46, wherein the adjuvant is a thrombolytic agent.

48. A method of claim 47, wherein the thrombolytic agent is tissue plasminogen activator.

49. A method of claim 46, wherein the adjuvant is an antagonist of a receptor chosen from the group consisting of: NMDA, AMPA, glycine, calcium channel and sodium channel.

50. A method of claim 45, wherein the B<sub>1</sub> agonist is chosen from these examples which can be found in the following citations: Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-272.

51. A method of claim 50, which further comprises administering an adjuvant.

52. A method of claim 38, wherein the cerebral ischemic injury is cortical ischemic injury.

53. A method of claim 52, wherein the B<sub>1</sub> agonist is chosen from these examples which can be found in the following citations: Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; 5 Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-272.

54. A method of claim 53, which further comprises administering an adjuvant.

10 55. A method to treat the reduction in the ability to perform fine motor skills due to neuronal disease in a patient in need of such treatment, comprising administering a B<sub>1</sub> agonist.

56. A method of claim 55, wherein the B<sub>1</sub> agonist is chosen from these examples which can be found in the following citations: Regoli and Basabe, *Pharmacological Review*, 1980, 32, 1-46; Marceau, *Immunopharmacology*, 1995, 30, 1-26; Audet, *et al. Pharm. and Ther.*, 1997, 280, 6-15; 15 Regoli, *et al., Can. J. Physiol. Pharm.*, 1977, 55, 855-867; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1993, 266, 192-199; Levesque, *et al., Immunopharmacology*, 1995, 29, 141-147; Drapeau, *et al., J. Pharm. Exp. Ther.*, 1991, 259, 997-1003; Drapeau and Regoli, *Methods in Enzymology*, 1988, 163, 263-272.

57. A method of claim 56, which further comprises administering an adjuvant.

20 58. A method of claim 55, wherein the neuronal disease is Parkinson's disease.

59. A method of claim 55, wherein the neuronal disease is Huntington's disease.

60. A method to treat reperfusion injury in a patient in need of such treatment comprising

administering a B<sub>1</sub> agonist.

**Figure 1**  
**Effect of the Bradykinin B2 Antagonist CP-0597**  
**on Total Infarct Volume in a Rat Model of Stroke**

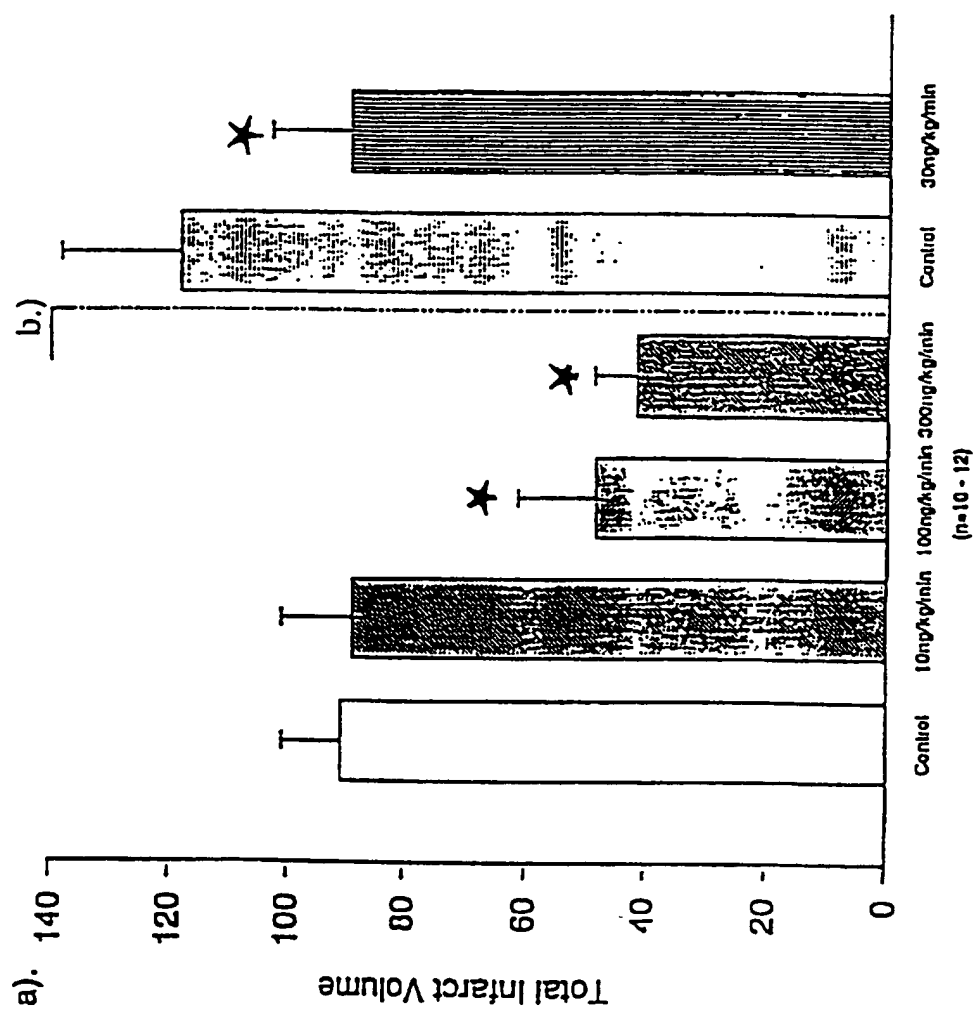


Figure 2  
Effect of CP-0597 on Striatal Infarct Size

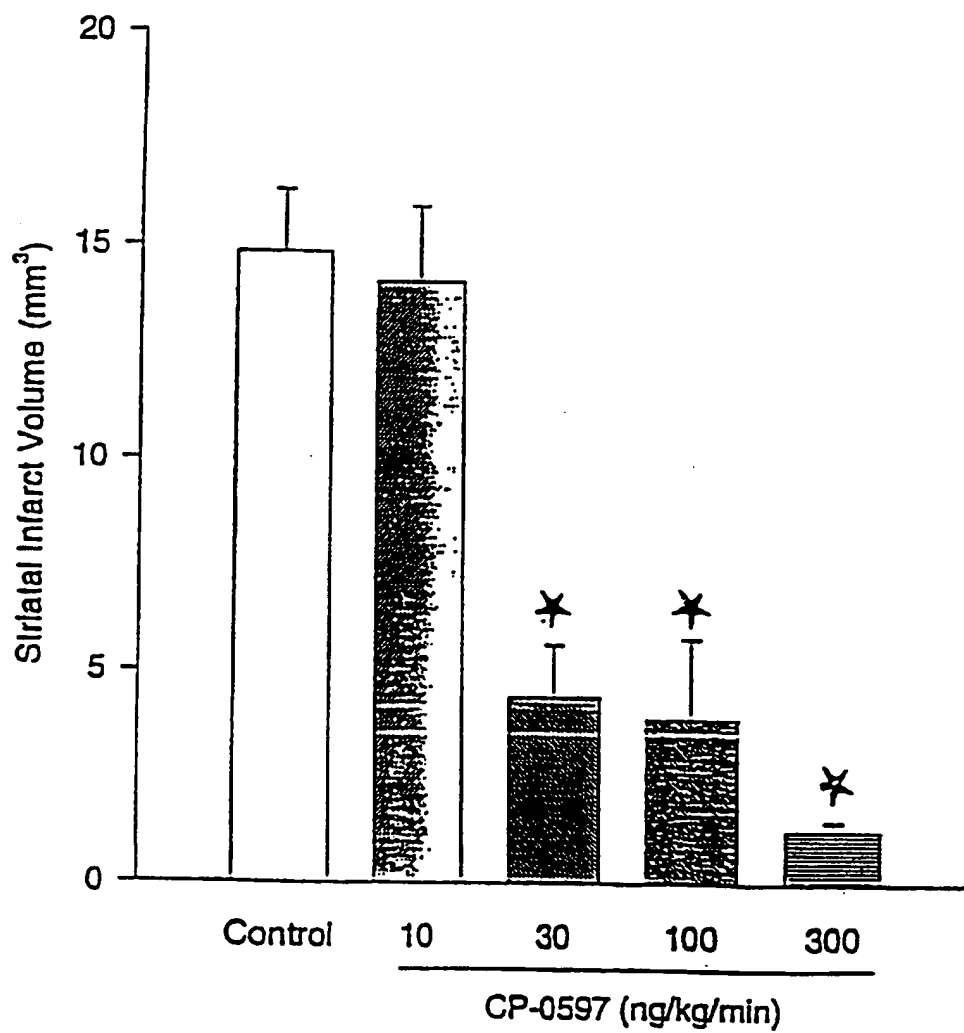
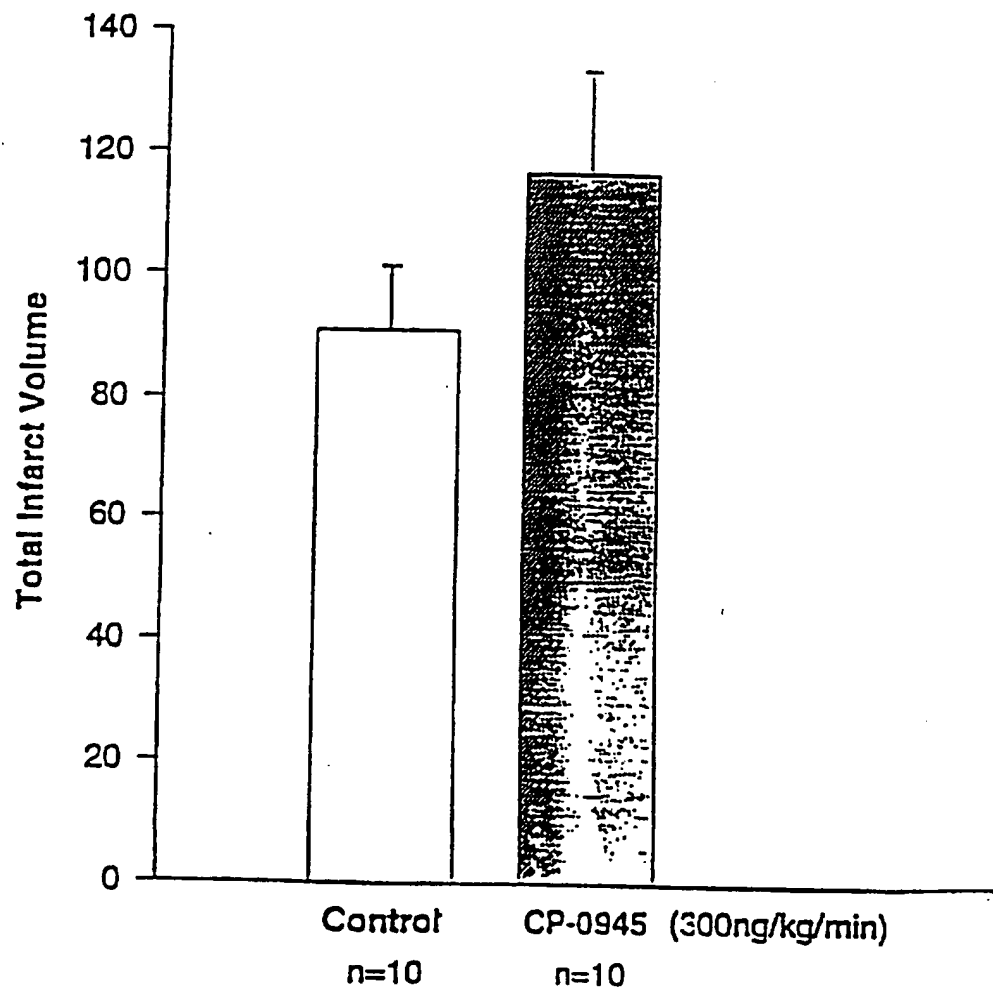
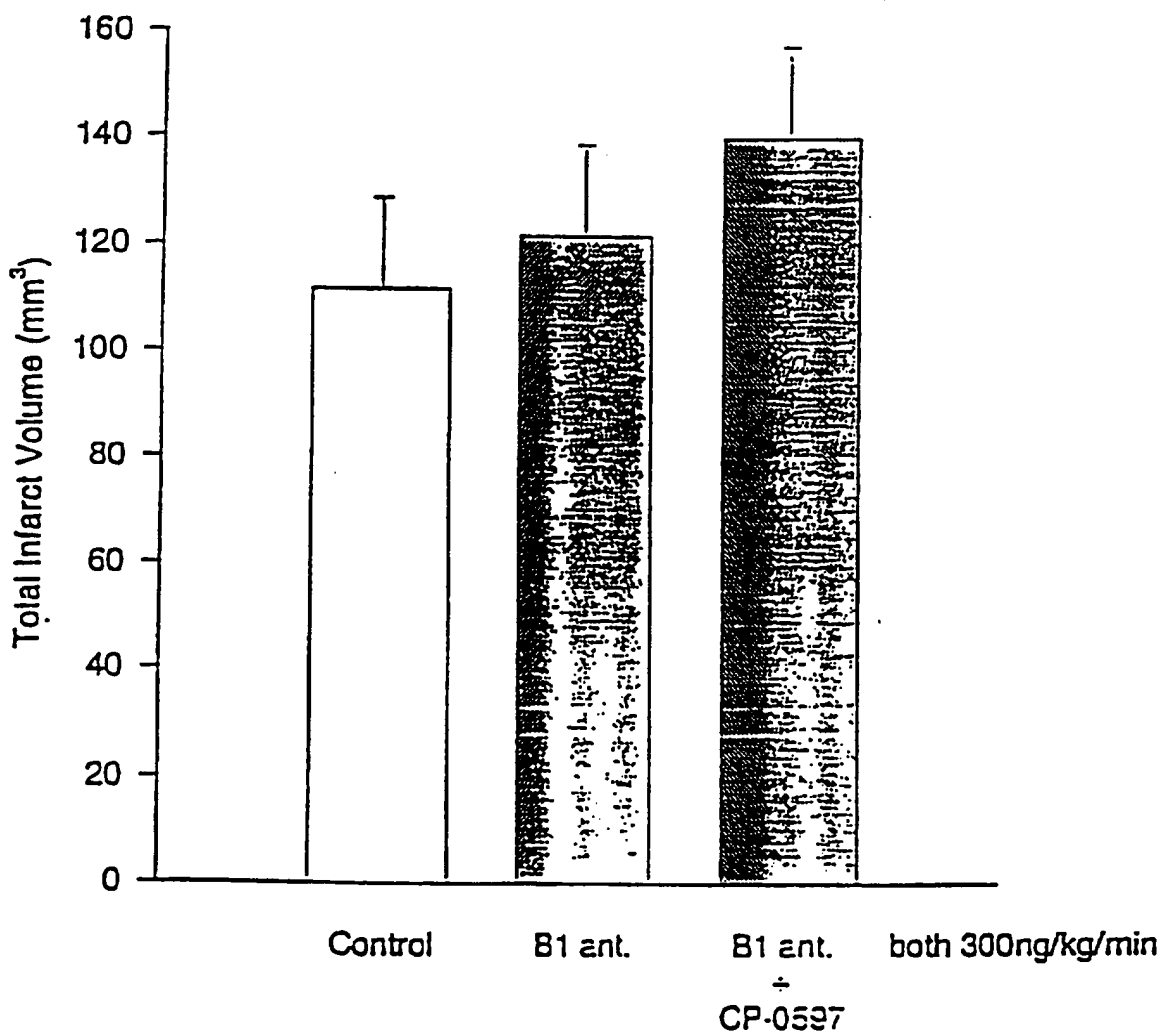


Figure 3  
Effect of the  $B_2/B_1$  Antagonist B9430  
on Total Infarct Volume in a Rat Model of Stroke





**Figure 4**  
**Effect of the B1 Antagonist B-9858 alone**  
**and in combination with the B2 Antagonist**  
**CP-0597 on total infarct volume**



n=10-12

Figure 5  
Effect of the B2 Antagonists HOE140 and  
NPC 17731 on total infarct volume

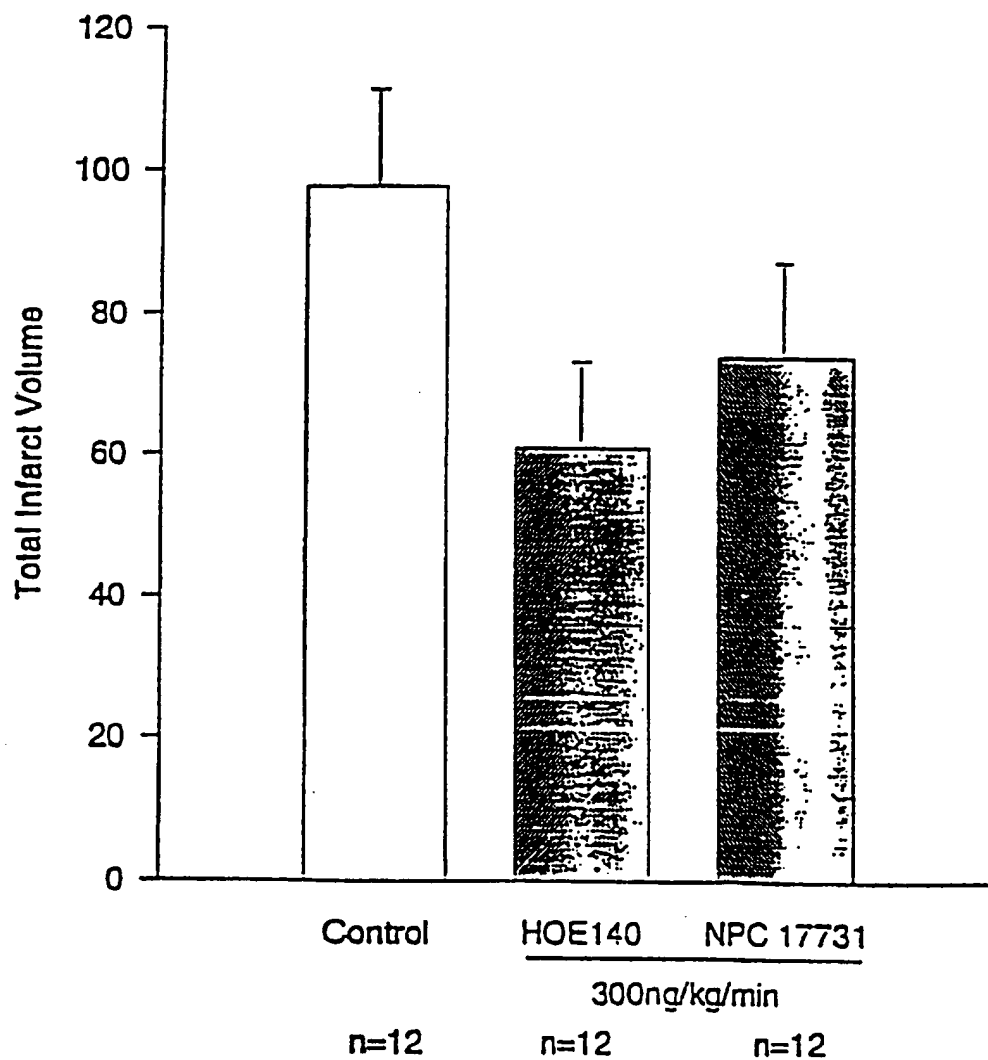
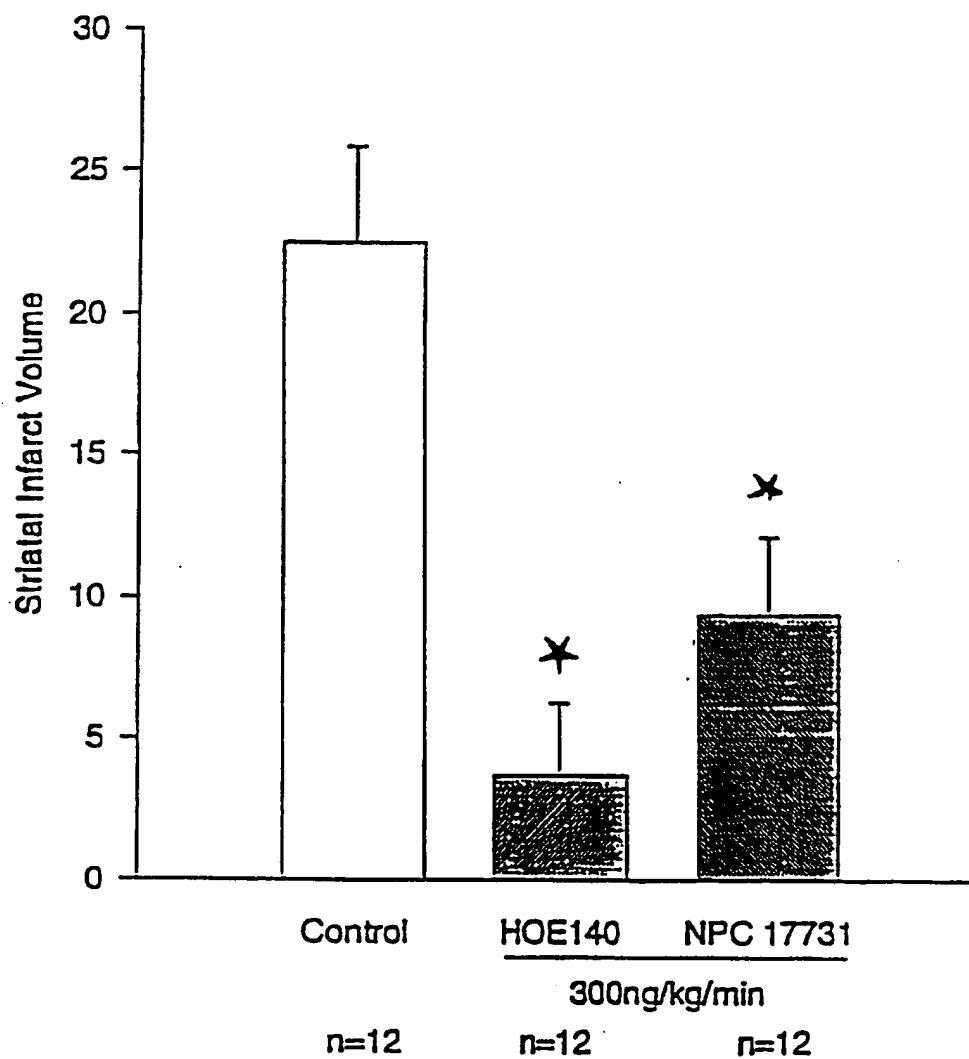
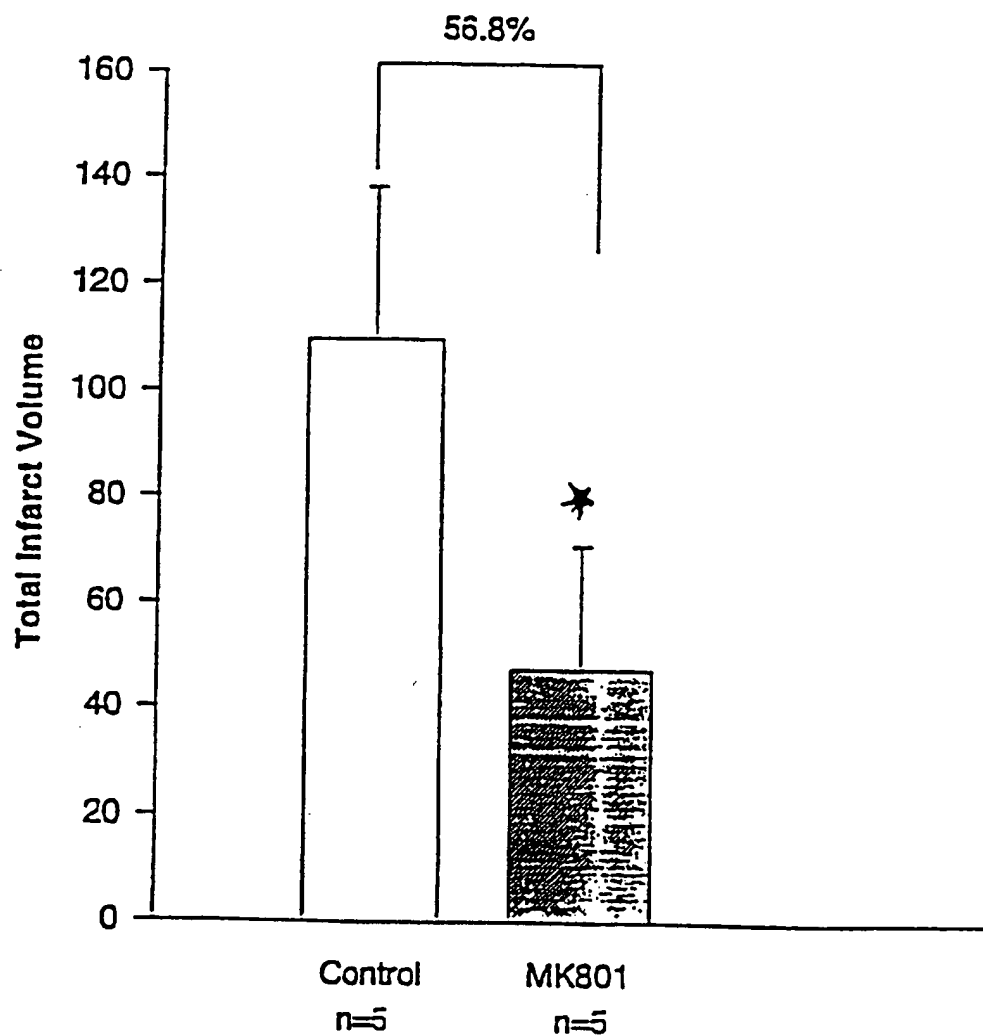


Figure 6  
Effect of the B2 Antagonists HOE140 and  
NPC 17731 on striatal infarct volume

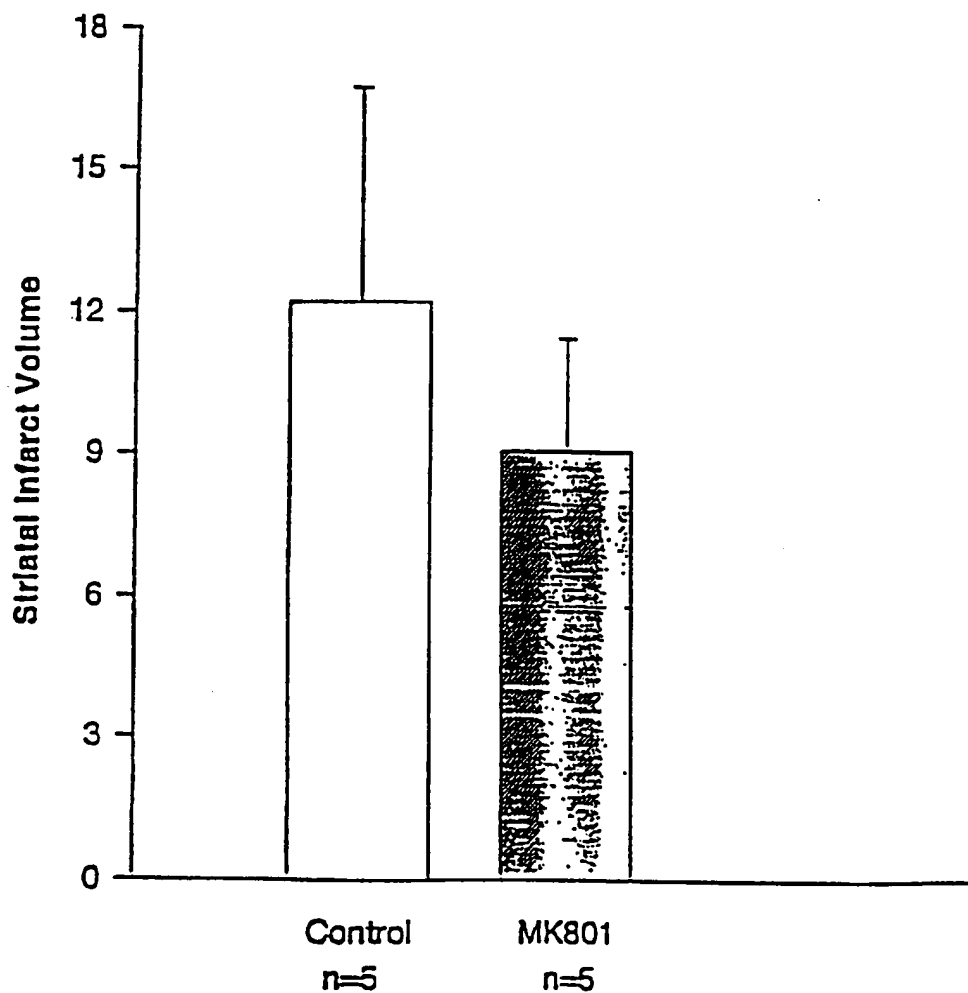


**Figure 7**  
**Effect of the NMDA Antagonist MK801 (4mg/kg s.c.) on**  
**Total Infarct Volume in a Rat Model of Stroke**



## Figure 8

Effect of the NMDA Antagonist MK801  
(8mg/kg s.c.) on Striatal Infarct Volume



**Figure 9a**  
**Effect of the B2 antagonist given post MCAO on**  
**Total infarct volume in a rat model of stroke**

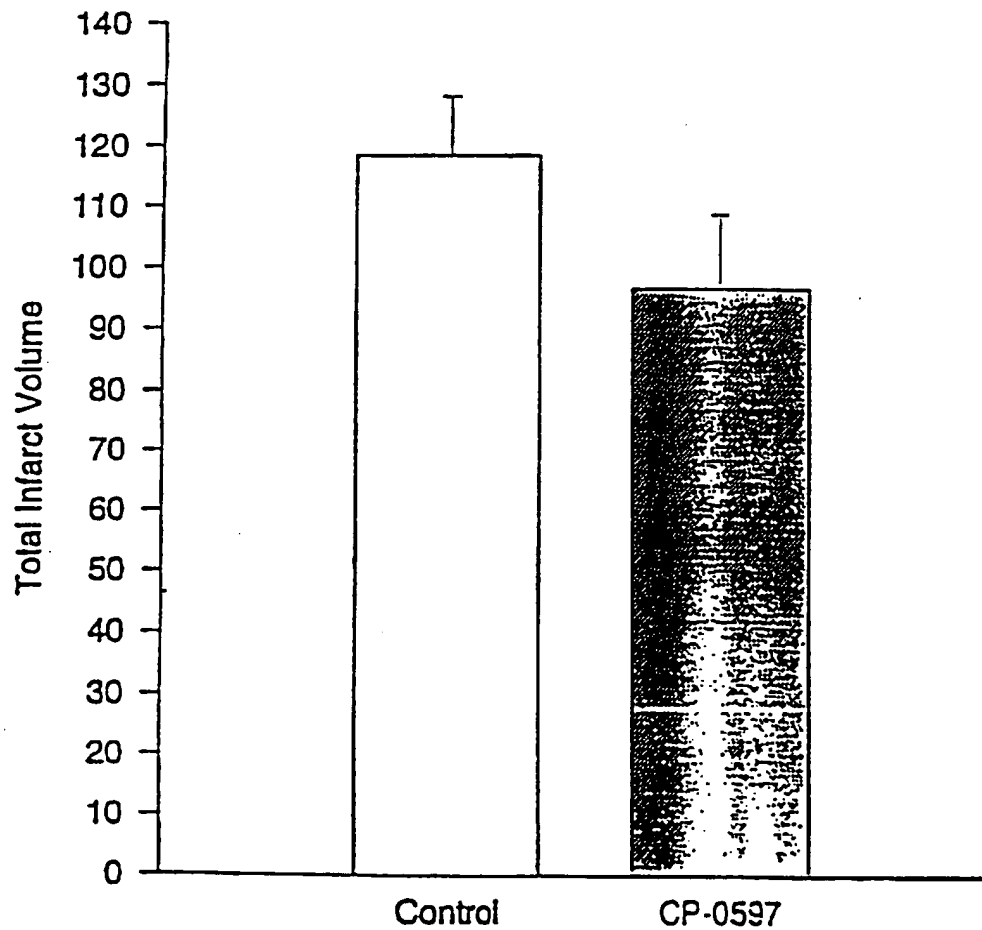
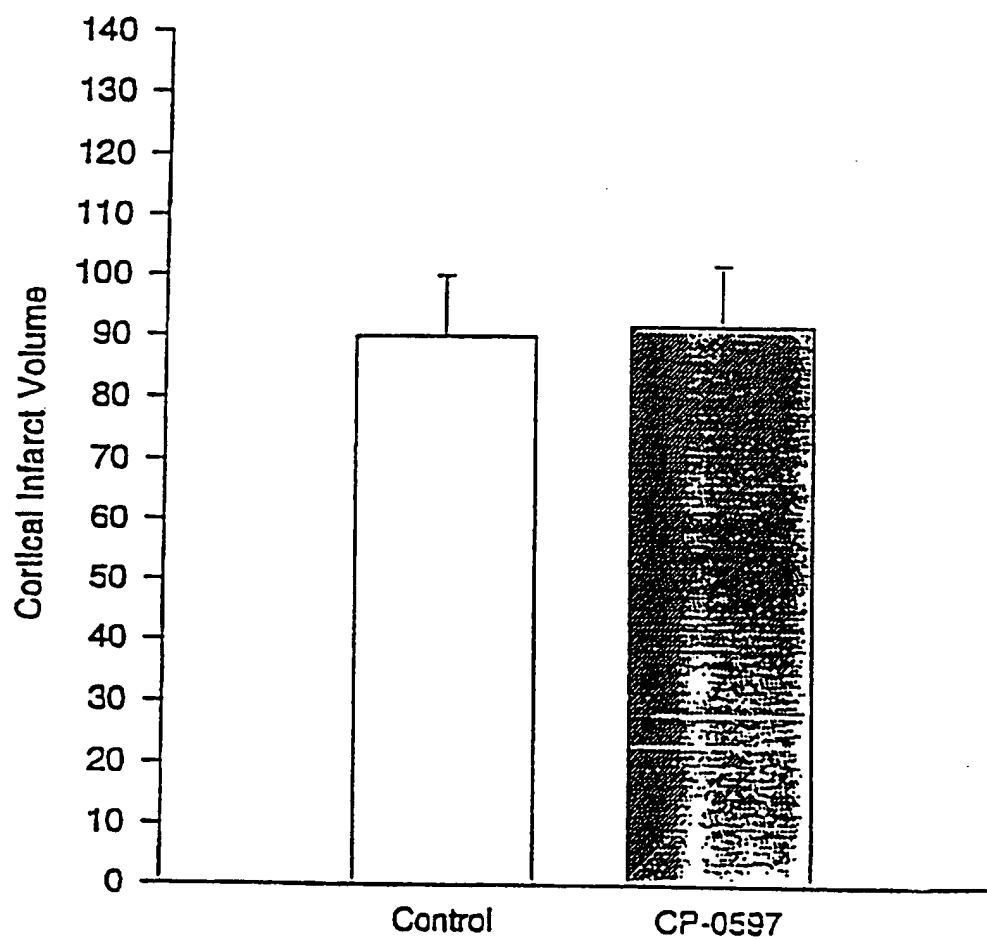
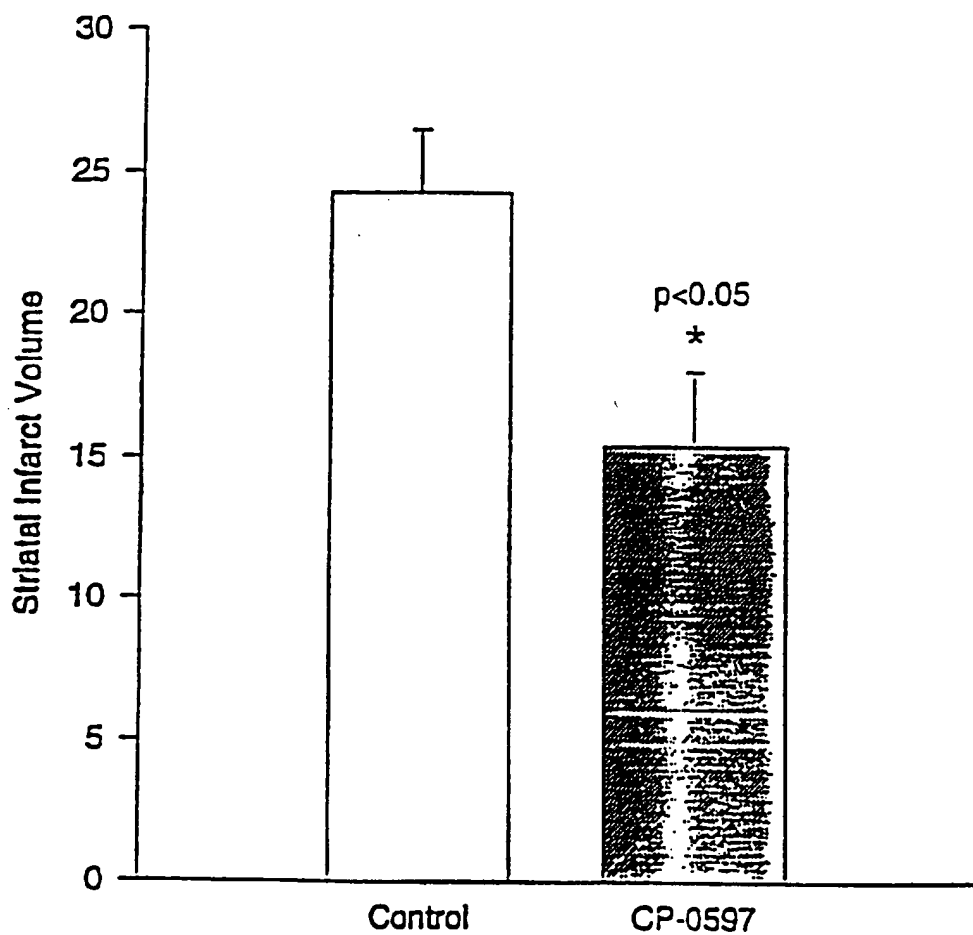


Figure 9b  
Effect of the B2 antagonist CP-0597 given post MCAO on  
Cortical infarct volume in a rat model of stroke



**Figure 9c**  
**Effect of the B2 antagonist CP-0597 given post MCAO on striatal volume in a rat model of stroke**





**Figure 10**  
***Structures of bradykinin B<sub>2</sub> antagonists***

---

HOE 140    D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg

CP-0597    D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-NChg-Arg

NPC 17731    D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-D-Hyp E trans propyl-Oic-Arg

Fig. 11

***Effect of the B<sub>2</sub> antagonist CP-0597  
on behavioral score before and  
after reversible MCAO in the rat***

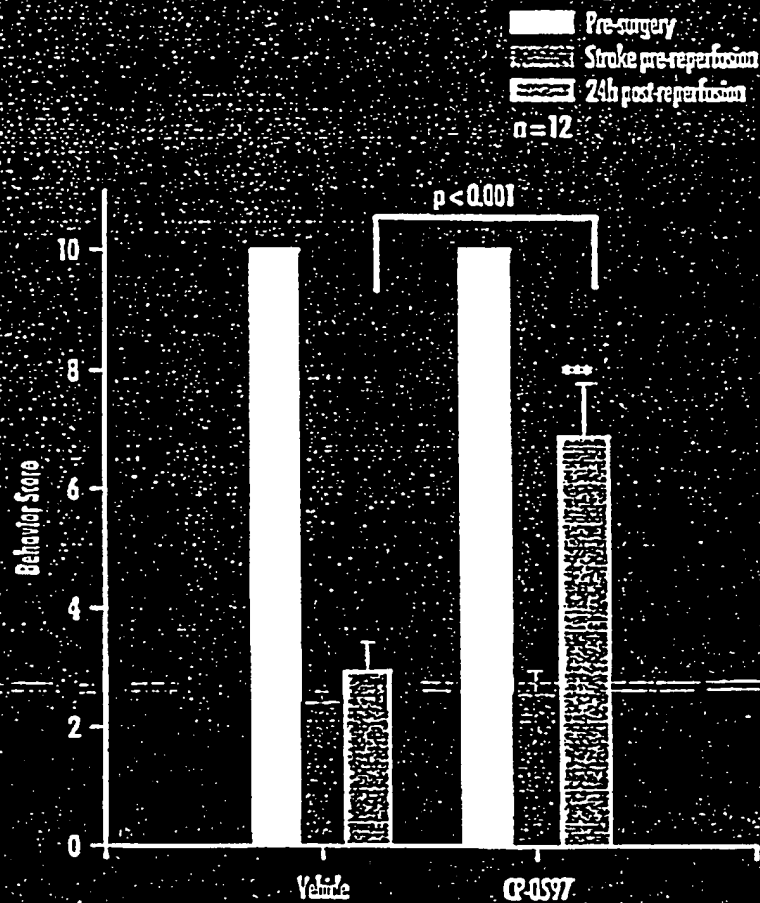
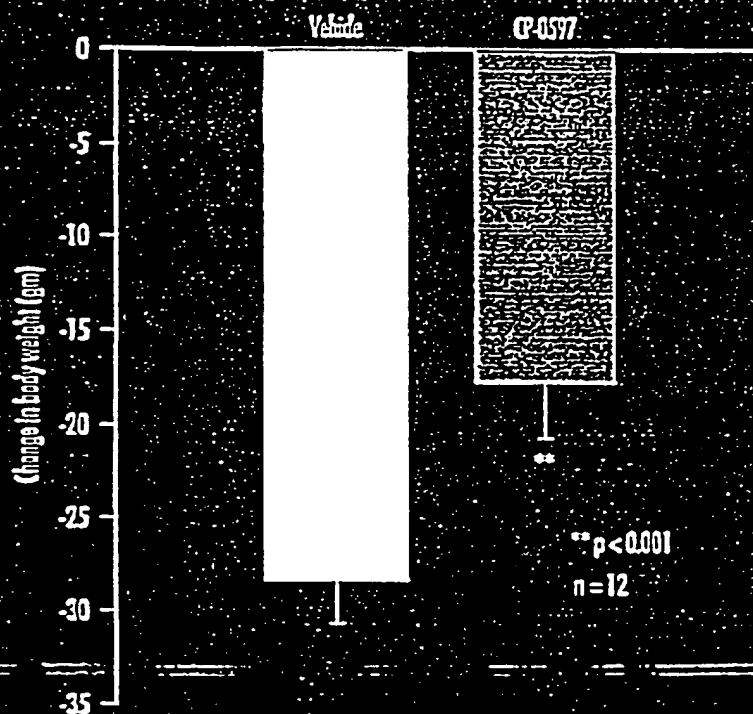
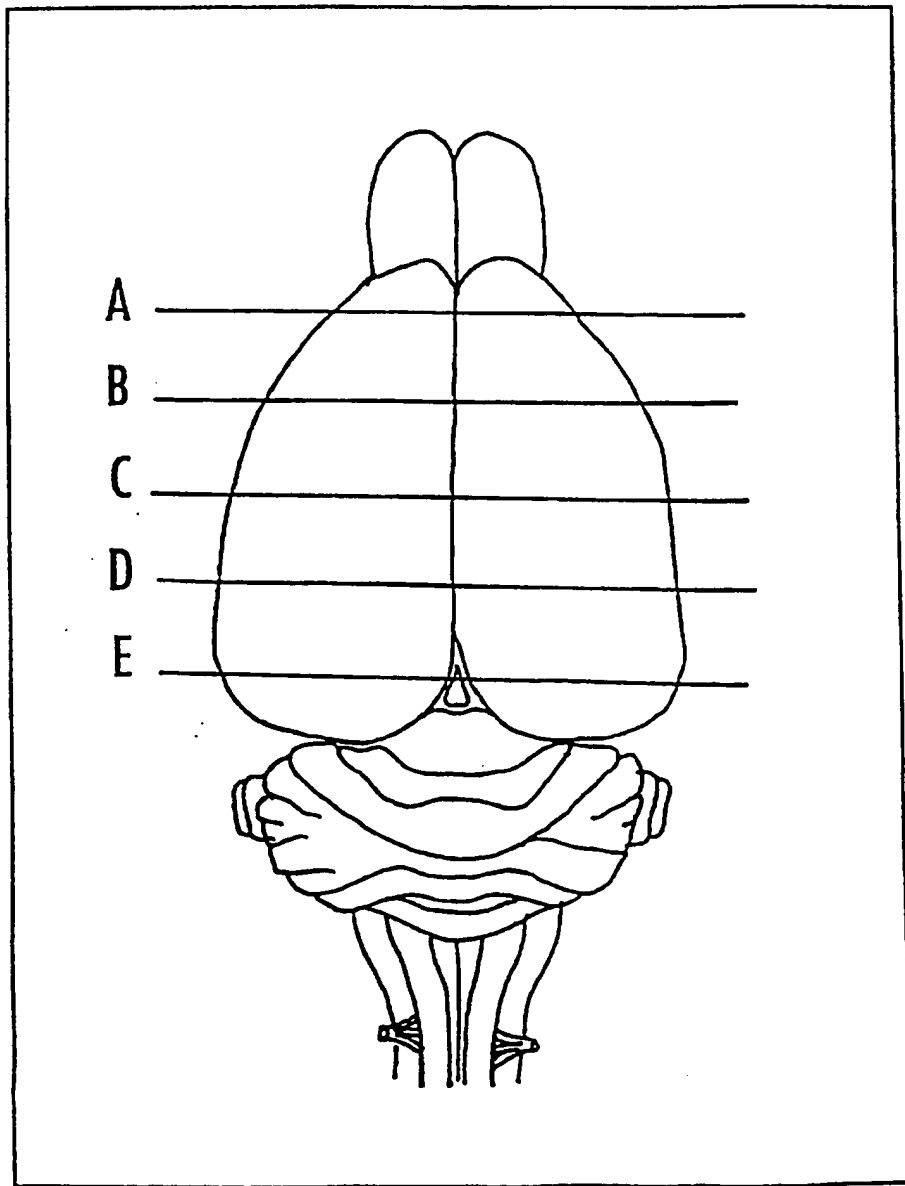


Fig. 12

***Effect of CP-0597 on change in body weight 24 hours after reversible MCAO in the rat***



*Fig 13*  
*Dorsal view of rat brain indicating  
sections used in the rMCAO model  
of stroke*



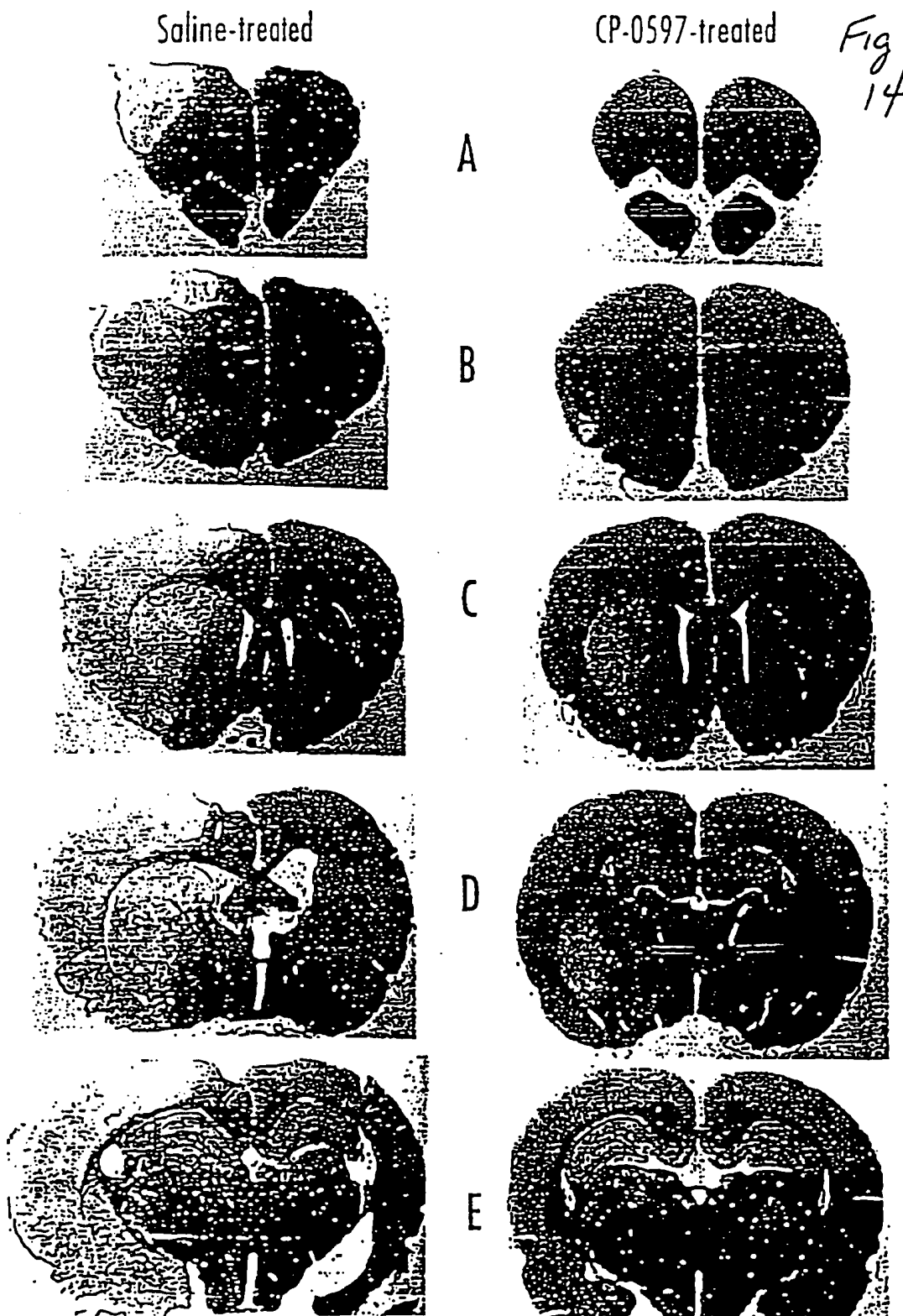
*Coronal sections of rat brain 24h post rMCAO*

Fig. 15

**Effect of CP-0597 on edema:**  
**—percent increase in hemisphere size**

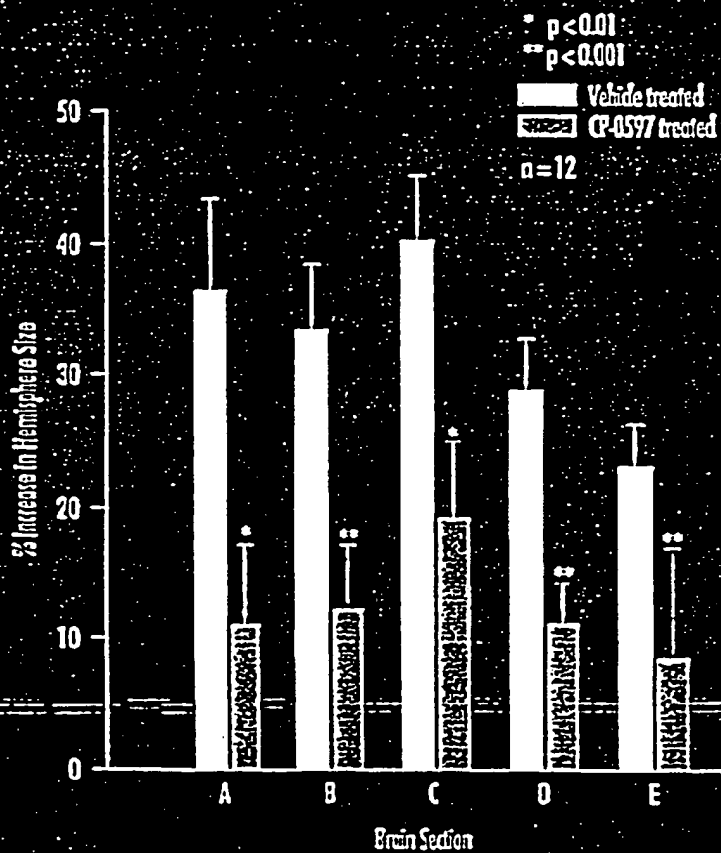


Fig. 16

# *Effect of CP-0597 on absolute infarct area 24 hours after rMCAO*

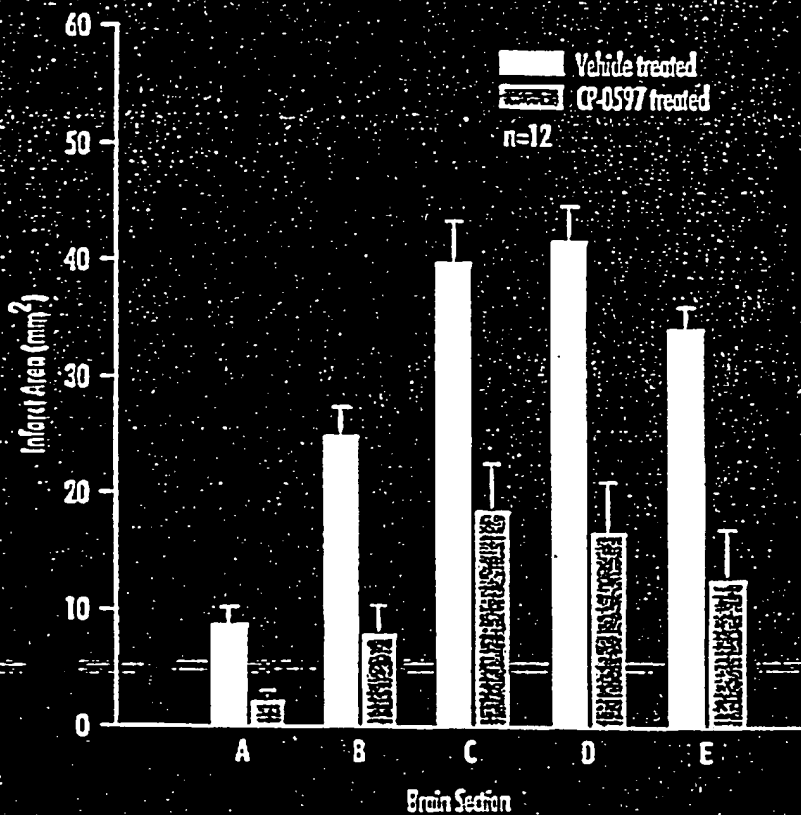


Fig. 17

# *Effect of CP-0597 on absolute infarct volume 24 hours after rMCAO*

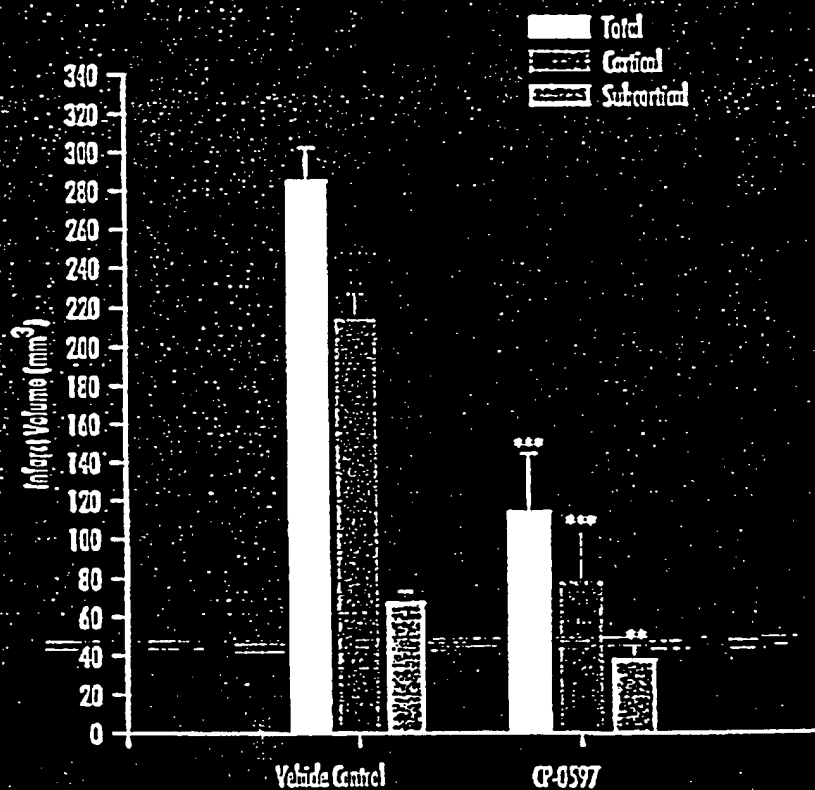




Fig. 18

# *Effect of CP-0597 on infarct area 24 hours after rMCAO*

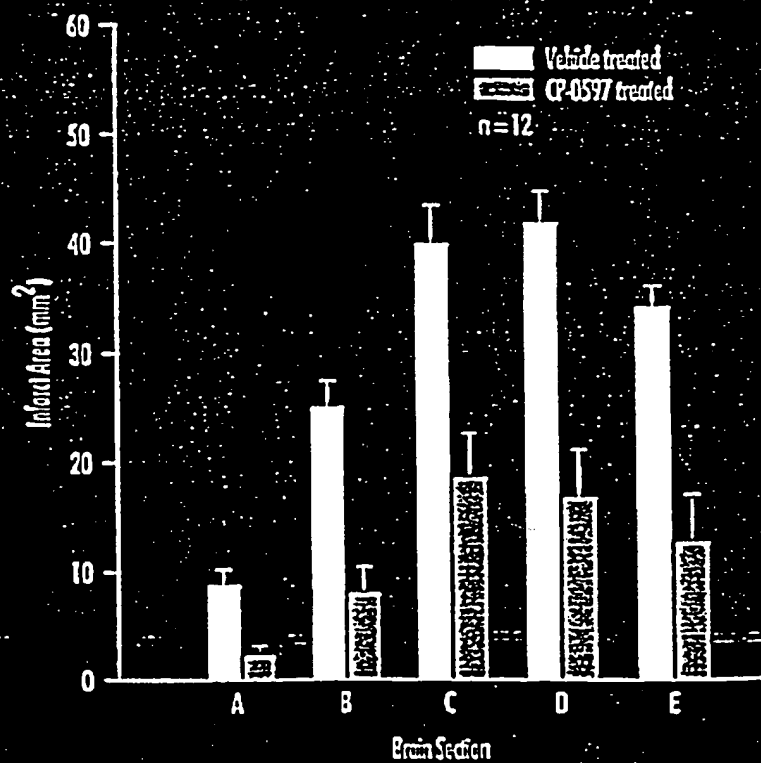
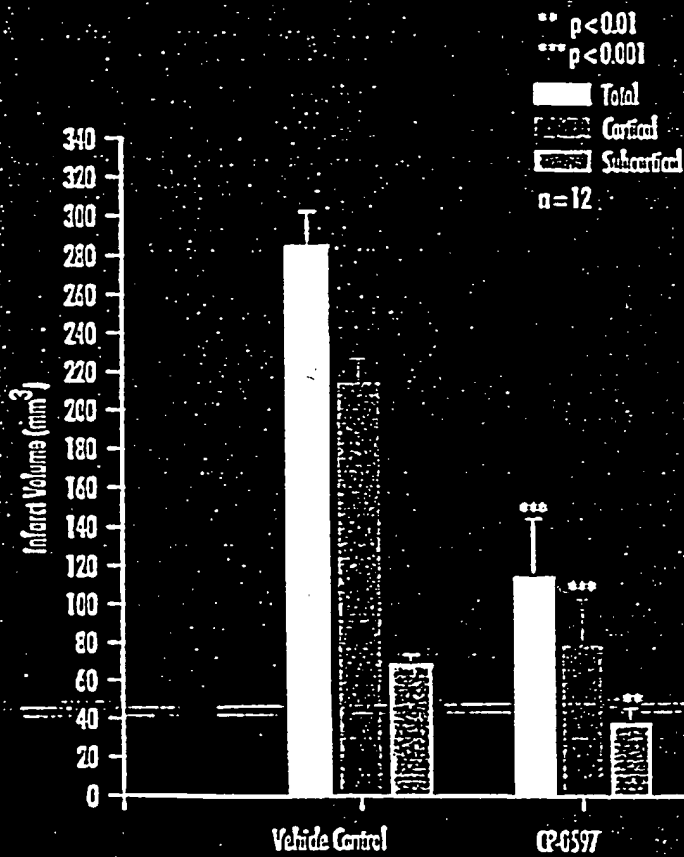
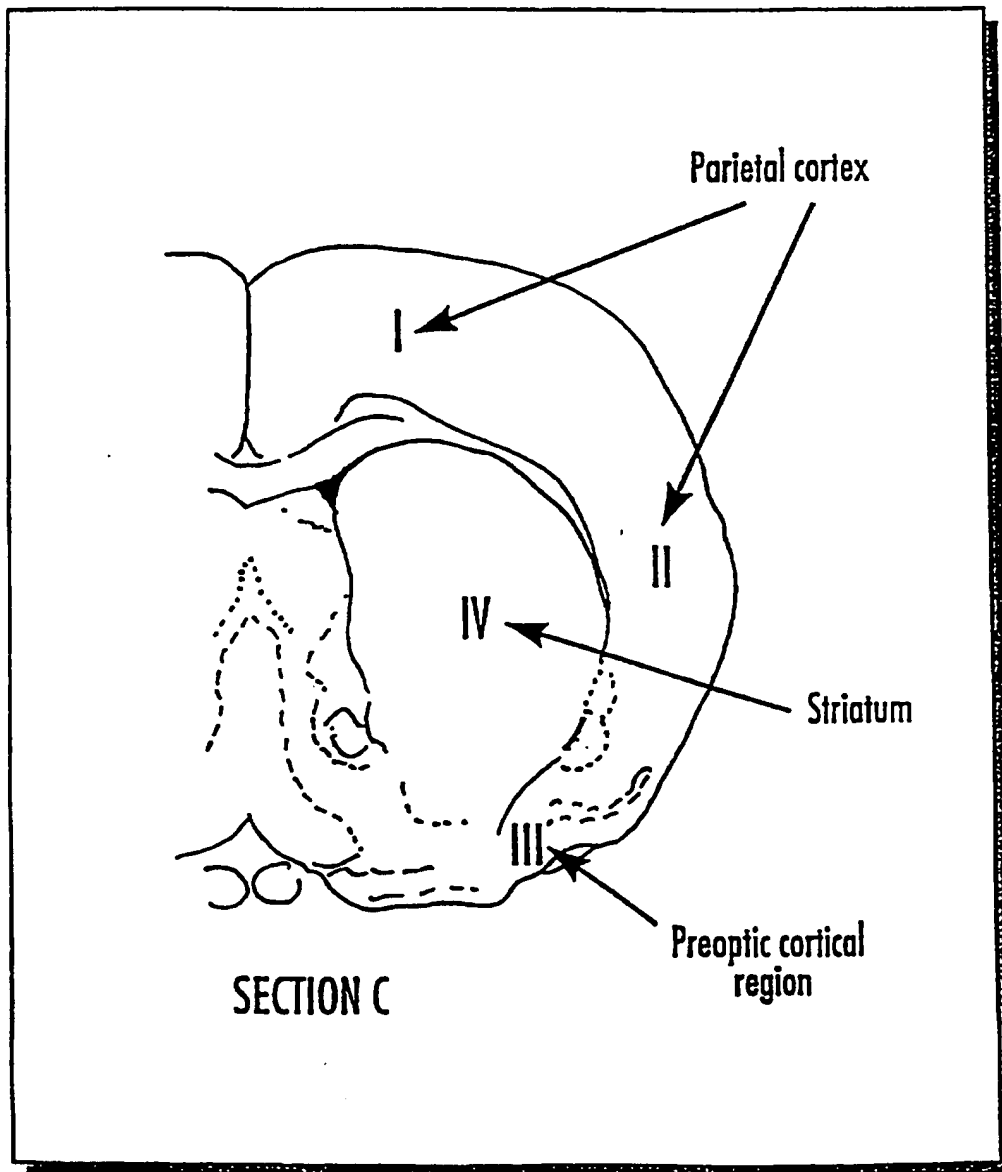


Fig. 19

## Effect of CP-0597 on infarct volume 24 hours after rMCAO



*Fig. 20*  
*Areas of rat brain sampled for*  
*neuronal damage*



# *Neuronal counts, Section C, Area II*

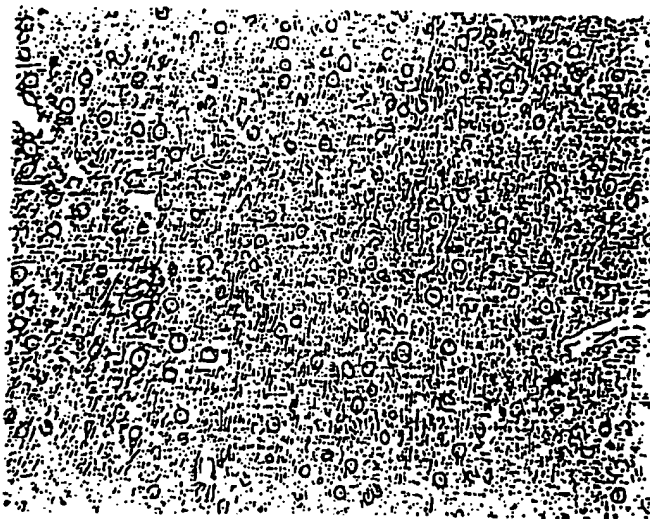
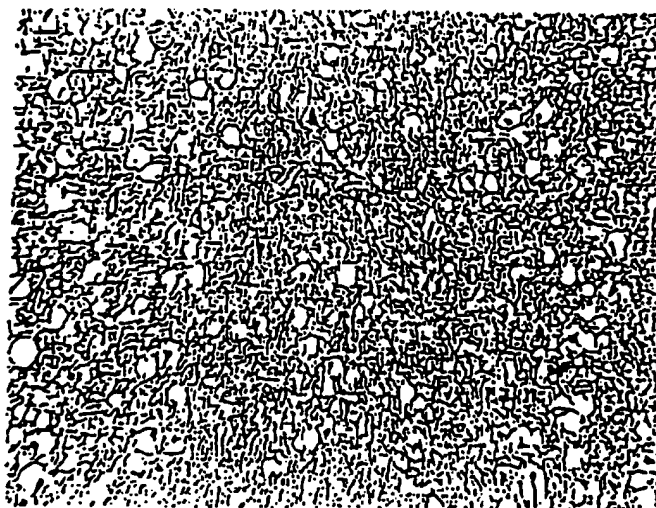
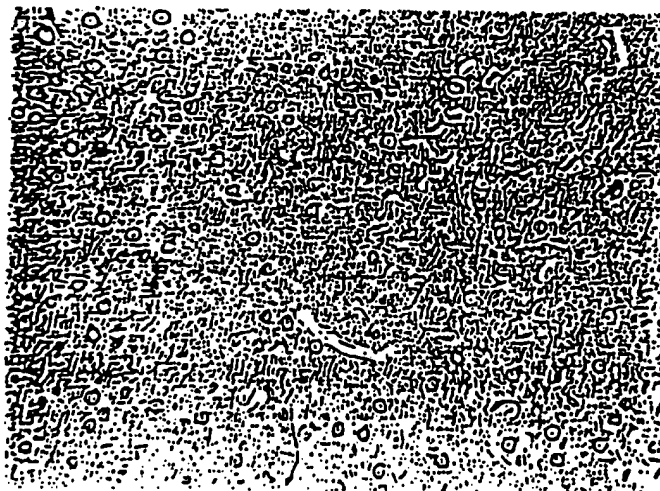


Fig  
21

Sham



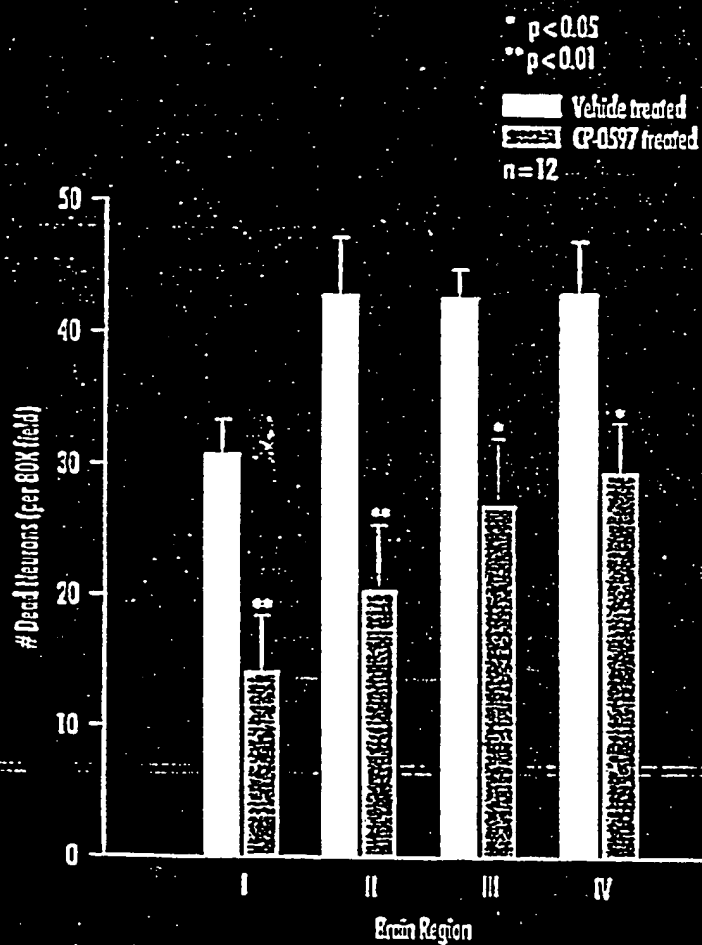
Saline-treated



CP-0597-treated

Fig. 22

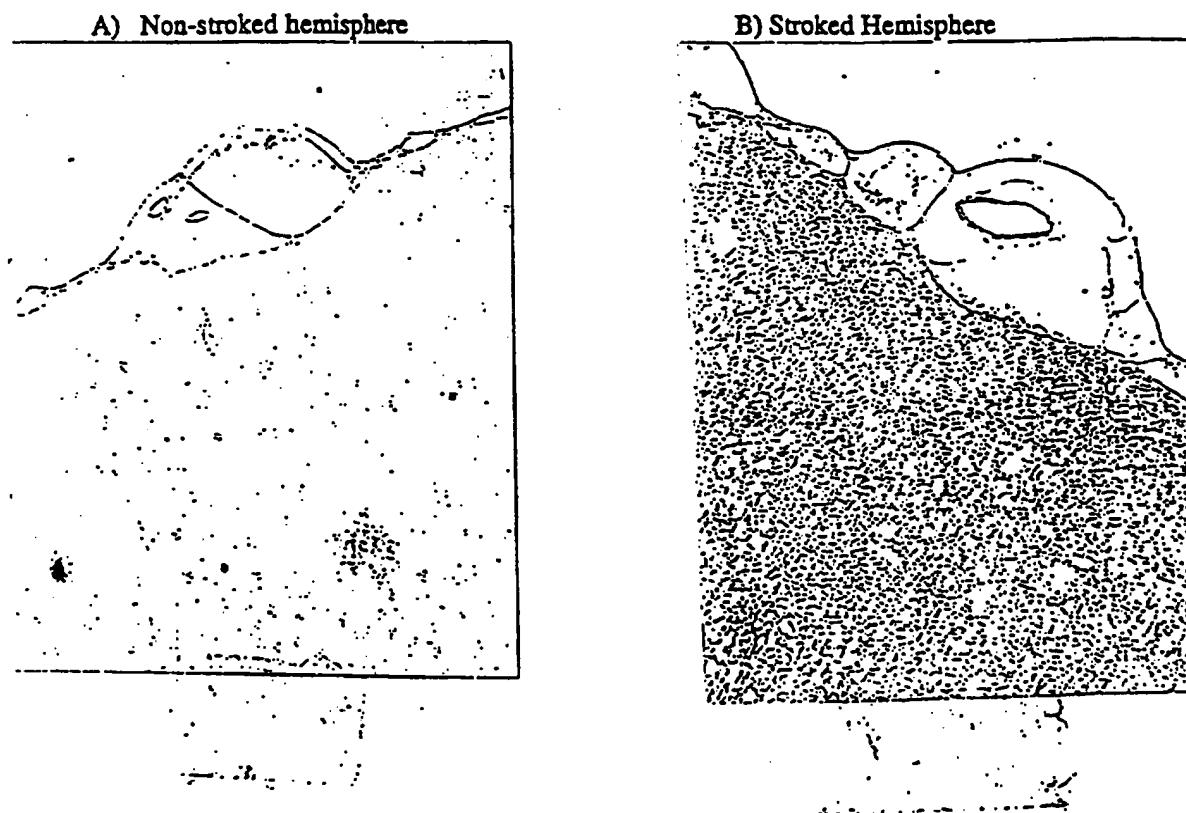
# *Effect of CP-0597 on number of dead neurons per 80X field*



**Figure 23**

Identification of bradykinin B<sub>1</sub> receptors, using a biotinylated B<sub>1</sub> ligand, in rat brain following 1 hour of ischemia and 24 hours of reperfusion. Note B<sub>1</sub> receptors (black stain) in B associated with specific neurons.

5



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/14201**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 38/00

US CL :514/14, 15, 16

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/14, 15, 16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

APS

HOE 140

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 5,610,142 A (MAVUNKEL et al.) 11 March 1997, column 1, line 30 to column 2, line 58.	1-60



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:		*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A	document defining the general state of the art which is not considered to be of particular relevance		
*B	earlier document published on or after the international filing date	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O	document referring to an oral disclosure, use, exhibition or other means		
*P	document published prior to the international filing date but later than the priority date claimed	*A	document member of the same patent family

Date of the actual completion of the international search

03 NOVEMBER 1997

Date of mailing of the international search report

19 NOV 1997

Name and mailing address of the ISA/US  
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